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PROCEEDINGS
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ORGANISMS REQUIRING VITAMIN B_1

By William J. Robbins

Department of Botany, University of Missouri

Communicated December 27, 1937

In earlier articles[1] from this laboratory we have reported that excised tomato roots required an external supply of vitamin B_1 or of thiazole[2] for continued growth. The pyrimidine alone was ineffective. *Phycomyces Blakesleeanus* was found to require vitamin B_1 or both intermediates.[3] Neither of the intermediates alone was effective. *Phycomyces nitens* and two species of Torula[4] which we have investigated, *Torula fermentati* and *T. Laurentii*, resemble *Phycomyces Blakesleeanus* in requiring an external supply of both intermediates for good growth.

We have found some organisms also which grow well in a medium supplemented with pyrimidine alone, with a mixture of pyrimidine and thiazole, or with vitamin B_1. The thiazole alone is ineffective. Among these organisms are *Torula rosea, T. sanguinea, Phytophthora fagopyri, Schizophyllum commune, Sclerotium delphinii, S. Rolfsii, Sphaerulina trifolii, Pythium Butleri* and *P. polycladon*.[5] In a liquid medium composed of 0.5% $MgSO_4.7H_2O$, 1.5% KH_2PO_4, 1% or 0.5% Asparagine, 5% or 10% dextrose and certain mineral supplements, these seven organisms grow poorly or not at all. The addition to this medium of 30 units[6] of pyrimidine, a mixture of 30 units of pyrimidine and 30 units of thiazole or of 30 units of vitamin B_1 permits good growth.

None of the organisms mentioned above can be said to require an external supply of vitamin B_1 since they grow well in a medium lacking vitamin B_1 but supplemented with one or with both of the intermediates.

We have assumed on the basis of evidence presented earlier[3] that the vitamin B_1 molecule is the effective agent in determining the growth of *Phycomyces*, and that this organism when grown in a mixture of pyrimidine and thiazole synthesizes the vitamin molecule from the intermediates. Schopfer and Jung[7] are inclined to believe that the intermediates function as such and that *Phycomyces*, for example, when grown in a solution containing vitamin B_1, splits the vitamin molecule into the intermediates which then play their respective rôles in the metabolism of the organism.

We have found that *Torula rosea*, *T. sanguinea*,[4] *Phytophthora fagopyri* and *Pythium Butleri*[5] synthesize thiazole when grown in a medium supplemented with pyrimidine only. Furthermore, excised tomato roots form pyrimidine in a solution supplemented with thiazole only. This shows that some organisms which grow in a medium supplemented with pyrimidine alone or with thiazole alone form the missing intermediate from the elementary substances in the medium. This does not demonstrate that the molecule of vitamin B_1 rather than its thiazole and pyrimidine intermediates is the effective agent. It is, however, a result which would be anticipated on the basis of our assumption.

If we are correct in assuming that the vitamin molecule, as such, is the effective agent it might be anticipated that some of the more highly parasitic fungi may not be capable of synthesizing the vitamin molecule from the intermediates. For such organisms it would not be possible to replace the vitamin by its intermediates. They would require for growth an external source of vitamin B_1.

We have found certain species of *Phytophthora* which apparently require vitamin B_1 and cannot utilize the intermediates satisfactorily. *Phytophthora cinnamomi* and *P. capsici* are of this type. In our experiments these two organisms grew well in a medium of mineral salts and sugar supplemented with vitamin B_1. In the absence of the vitamin or with thiazole, pyrimidine or a mixture of the thiazole and pyrimidine we have secured little or no growth. It is possible that the synthesis of the vitamin from its intermediates is enzymatic and that these organisms lack the necessary enzyme.

Sinclair[8] and Schopfer and Jung[7] also have found that the two intermediates will replace the vitamin for *Phycomyces Blakesleeanus*. Neither intermediate alone is effective. Schopfer[9] has reported that *Rhodotorula flava* and *Rhodotorula rubra* are organisms which grow with pyrimidine alone, the thiazole alone is ineffective; and that *Mucor Ramannianus*[10] requires thiazole but not pyrimidine. We are inclined to believe on the basis of the evidence now available that the vitamin molecule is necessary for all of these organisms. Some kinds are capable of synthesizing from the elementary constituents of the medium sufficient for good growth. Others are not. Some synthesize enough of one of the intermediates for good growth but under the conditions of our experiments must be supplied with the other; some synthesize neither of the intermediates in amounts adequate for normal growth but are able to utilize the intermediates if supplied; and some not only do not synthesize either intermediate but are incapable of utilizing them when they are supplied.

The different groups of organisms in their relation to vitamin B_1 and its intermediates are summarized in the following table. The + sign indicates a positive growth effect of the substance given; the − sign, little or no effect.

ORGANISMS	THIAZOLE	PYRIMIDINE	THIAZOLE AND PYRIMIDINE	VITAMIN B_1
Group I	−	−	−	+
Group II	−	−	+	+
Group III	−	+	+	+
Group IV	+	−	+	+

In Group I are included those organisms which require an external supply of vitamin B_1; for example, *Phytophthora cinnamomi* and *P. capsici.*

Group II includes organisms which require for good growth an external supply of vitamin B_1 or of both intermediates. The thiazole alone or the pyrimidine alone is ineffective. Examples are *Phycomyces Blakesleeanus, Phycomyces nitens, Torula Laurentii* and *T. fermentati.*

Group III includes those which require an external supply of vitamin B_1, of both intermediates or of pyrimidine. The thiazole alone is ineffective. Examples are *Phytophthora fagopyri, Pythium Butleri, P. polycladon, Schizophyllum commune, Sclerotium delphinii, Sclerotium Rolfsii* and *Sphaerulina trifolii.*

Group IV includes those which require an external supply of vitamin B_1, of pyrimidine and thiazole, or of thiazole. The pyrimidine alone is ineffective. Examples are *Mucor Ramannianus* and excised tomato roots.

A fifth group might be included comprising organisms which are unaffected by the vitamin or its intermediates in amounts which are effective for the organisms given above. This group includes many saprophytic organisms, for example, *Aspergillus niger,* and perhaps some parasites.

The members of a sixth group (*Rhizopus nigricans*)[5] are inhibited by amounts of the vitamin or of its intermediates which are favorable for other organisms.

These observations suggest that a biological method of detecting the presence of the vitamin or of its intermediates could be devised. Its success would depend upon the use of suitable organisms and further evidence of the specificity of vitamin B_1 and its intermediates for the organisms. It would also depend in part upon the presence of the vitamin (or intermediates) within certain limits, and the absence of injurious material which would inhibit the growth of the organisms. The scheme presented below has not been used on natural materials and is suggestive only.

I *Phytophthora cinnamomi*
Positive growth effect = vitamin B_1
No growth effect = both intermediates or pyrimidine alone or thiazole alone or no vitamin B_1 nor intermediates See II

II *Phycomyces Blakesleeanus*
Positive growth effect = both intermediates
No growth effect = pyrimidine alone or thiazole alone or no vitamin B_1 nor intermediate See III

III *Pythium polycladon*
Positive growth effect = pyrimidine alone
No growth effect = thiazole alone or no vitamin B_1 nor intermediate See IV

IV Tomato root or *Mucor Ramannianus*
Positive growth effect = thiazole alone
No growth effect = no vitamin B_1 nor intermediate

[1] William J. Robbins and Mary A. Bartley, *Sci.*, **85**, 246–247 (1937). William J. Robbins and Mary A. Bartley, *Proc. Nat. Acad. Sci.*, **23**, 385–388 (1937).

[2] When the terms, thiazole or pyrimidine, are used in this paper, the 4-methyl-5-hydroxyethylthiazole or the 2-methyl-5-bromo-methyl-6-aminopyrimidine is meant. These intermediates have been used in the synthesis of vitamin B_1.

[3] William J. Robbins and Frederick Kavanagh, *Proc. Nat. Acad. Sci.*, **23**, 499–502 (1937).

[4] William J. Robbins and Frederick Kavanagh, *Plant Physiol.* (in press).

[5] William J. Robbins and Frederick Kavanagh, *Am. Jour. Bot.* (in press).

[6] A unit is 10^{-9} Mole of the compound in question.

[7] William Henri Schopfer and Albert Jung, *Compt. Rend. Acad. Sci., Paris*, **204**, 1500–1502 (1937).

[8] H. M. Sinclair, *Nature*, **140**, 361 (1937).

[9] William H. Schopfer, *Compt. Rend. Acad. Sci., Paris*, **205**, 445–447 (1937).

[10] Werner Müller and William Henri Schopfer, *Ibid.*, **205**, 687–689 (1937).

A CYTOLOGICAL STUDY OF COLCHICINE EFFECTS IN THE INDUCTION OF POLYPLOIDY IN PLANTS

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Communicated January 12, 1938

A series of colchicine treatments of plant tissues was begun by the writer as an independent investigation in February, 1937, while employed at the Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, N. Y. In a report at the annual winter meeting of the A. A. A. S. in 1936, Allen[1] mentioned that colchicine influenced mitotic activity in animal tissue. Soon thereafter Mr. E. L. Lahr,[2] a colleague in the

Department of Genetics, showed the writer some preparations of animal germinal tissue treated with colchicine previous to killing, in which mitotic figures were more abundant than in the prepared sections from untreated tissue. This observation suggested many possibilities.

It was recalled that Kostoff[3] working with amphidiploids stated that he had employed various chemical substances in treating plant tissue. His work and similar studies by others had been reviewed by the writer in a thesis[4] submitted several years previously. With these investigations as a background and an active interest in a polyploid series of plants, the Resedaceae,[5] it was natural for the writer to have an interest in any substance which might seem to offer the possibility of affecting the mitotic process. A small quantity of colchicine was obtained and a series of more or less successful preliminary experiments with plant tissues was begun.

Of course, onion root tips, radish and corn seedlings, with large nuclei and readily stainable chromatin offered distinct advantages for this type of investigation, so that most of the preliminary work up to the end of April, when the writer left Cold Spring Harbor, was done with these materials.

As the work progressed the main objectives of this initial cytological study using colchicine became centered around the following points: (1) the effect of colchicine upon individual embryonic cells rather than entire tissues; (2) the degree of polyploidy in a cytologically changed nucleus and the relation of the change to the method of treatment; (3) observable cytological changes due to the effects of colchicine upon the mitotic process; (4) the probable effectiveness of colchicine as a means of producing hereditary changes.

Since the cell reacts to the colchicine environment, independent of adjacent cells, it must be studied as an independent functional and structural unit. Therefore it is vital to consider the cytological effects upon individual embryonic cells of stem and root meristems.

Stem and root meristems of germinated *Zea mays*, *Raphanus sativa* and bulbs of *Allium cepa* were used to study the effect of colchicine upon their embryonic cells. The production of cytogenetical changes was found to be dependent upon three factors of the treatment, namely: (*a*) the concentration of colchicine solution, (*b*) the time allowed for the solution to act upon the meristem and (*c*) the physiological activity of embryonic cells at the time of treatment.

It is well known that embryonic cells are characterized by their ability to undergo mitosis and produce new cells. When meristematic cells divide mitotically the chromosomes organized from chromatin undergo equational longitudinal division into daughter chromosomes; these daughter chromosomes are completely separated from each other in metaphase, migrate to opposite poles in anaphase and subsequently reorganize as daughter

nuclei. It is also well known that the mitotic spindle formed during the process functions in the separation of daughter chromosomes and plays an important rôle in the formation of a cell plate between the daughter nuclei. The cell plate marks the place where the new cell wall is laid down to separate the two daughter cells and thus completes the mitotic process. The two new cells are genetically similar to each other and to the original parent cell. Interference or interruption of any one phase or several phases of the mitotic process could bring about the production of cells that differ from each other or from the parent cell. The colchicine treatments interrupted or inhibited certain phases of the mitotic process and without apparently affecting certain others.

Colchicine is soluble in water and effective in very dilute solutions. The solutions used in these original studies were in concentrations of 1%, 0.1% and 0.01% and applied for 24, 48, 72, 96 and 108 hours. The seeds were germinated upon moist filter paper soaked with the desired concentration of colchicine for a given interval of time. *Allium* bulb root tips were treated by immersion. Definite toxic effects were observed from the highest concentrations. An increase in colchicine concentration or an increase in length of time for treatment beyond 72 hours was found to be detrimental and fatal to the future development of the meristematic tissue. There was a close correlation between the production of morphological abnormalities and the intensity and duration of the treatment. Increase in concentrations and increase in time for treatment caused increases in both structural abnormalities and cytological abnormalities.

Stem and root meristems were analyzed for cytological changes by two methods, namely, the aceto-carmin smear method, and the paraffin method for preparation of permanent slides. A weak Flemming solution was used for fixation and several different cytological stains were used for staining the sections. The principal plants used in this study were *Allium cepa*, *Raphanus sativa*, *Zea mays* and *Triticum vulgare*. The general morphological and tissue changes which appeared in these stem and root meristems were all similar in character. Since *Allium* root meristems offered material with large nuclei which were of advantage for cytological study this form was used most extensively for the detailed cytological studies given here.

A 0.1% and 0.01% colchicine solution both produced enlargements of *Allium* root, and stem portions, figures 1b, 2b, 3b, were consistently larger than untreated material, figures 1a, 2a, 3a. A decrease in the concentration of colchicine solution increases the length of time necessary for production of the enlargement. A fairly concentrated solution of 1% has

EXPLANATION FOR FIGURES

Figure 1.—Treated and untreated *Allium*. Figure 2.—*Zea mays*. Figure 3.—*Raphanus sativa*. Figures 8, 22, 31 and 36, *Zea mays*. Other figures *Allium*. Cytological figures Mgn. × 650.

a
1
b
a
2
b
a
3
b
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6
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8
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10 11 12 13 14 15 16
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31
32
33
34
a
35
b
a
36
b

only a toxic effect, it does not produce enlargement even though the treatment is applied for sufficient time to produce change. The first sign of enlargement was found in the region of elongation some distance behind the dividing embryonic cell region. As the enlargement increased and time of treatment was prolonged, the bulbous portion came closer to the tip of the root.

This observation of enlargement over several days showed that as the swelling continued it approached the very tip of the root, and a similar progression of vascular elements were differentiating much nearer to the tip in treated roots, figures 35b, 36b, than in untreated root tips, figures 35a, 36a.

A section through one of these roots, figures 35b, 36b, shows this same progression in the differentiation of the vascular elements as compared with the untreated root tips, figures 35a, 36a.

The treatment is specific for individual cells, producing one type of change in a particular cell while the neighboring cell may be affected differently. This cytological variation from cell to cell was a characteristic feature. Such phenomena afford a basis for sectoral mutation as root tips and stem tips differentiate from the growing point.

The relationship of individual cellular reaction to the effects of colchicine, independent of adjacent cell activity is correlated with our knowledge of the individuality of mitotic activity found restricted to the activity of the cell and not the entire tissue. The enlargement of the tissue as a member is caused by increase in cell size rather than increase in cell numbers.

The chromosomes undergo longitudinal equational division, shown by a study of isolated chromosomes in aceto-carmin smear preparations, figures 10–16, which were made from colchicine treated root meristems of *Allium*. Colchicine does not inhibit or interfere with this process of chromosome division. The separation of the chromosome halves was complete except for a proportion of the achromatic region which seems to be associated with the constrictions of chromosomes. This phase of the mitotic process (longitudinal equational division of the chromosomes) must occur to produce polyploid nuclei, and during or after a moderate colchicine treatment this process continues without interruption.

The mitotic spindle or the formation of an achromatic figure was definitely inhibited in colchicine treated material as shown by chromosomes plates in aceto-carmin preparations of *Allium* in figures 5, 6, 7, 17 and 19. A paraffin section which was specifically prepared with weak Flemming solution, figure 34, and stained, with the triple stain, failed to show spindle formation. Similar material untreated and fixed in Flemming solution, and similarly stained, showed the presence of the spindle and chromosomes in a definite equatorial plate, figure 32, and a later mitotic

stage, figure 33, showed development of the spindle and cell plate between nuclei. From this and similar observations it was concluded that colchicine prevents spindle formation.

The diploid number of chromosomes for *Allium* is 16, shown in figure 4 from an untreated control. Counts of chromosome numbers in *Allium* after 48 hours of treatment with colchicine were 32 in figure 5; 48 in figure 6; 64 in figure 7. For the treated *Zea mays* stem meristems, figure 8, 40 chromosomes were counted. Application of pressure to the cover slips of aceto-carmin preparations made it possible to count the higher numbers. The evident cytological change was one of polyploidy. Polyploid cells could be found in both stem figures 8, 22, and root meristems, figures 5, 7, following colchicine treatment. Polyploidy of cells in the meristems of stems as well as root tissue indicates that colchicine may be valuable for the induction of polyploidy with practical significance, because these cytogenetical variations produced in the stem and transmitted by growth to the reproductive organs could be used for purposes of propagation and plant breeding.

Up to April the writer's investigation had not progressed beyond the cytological observations given in the paper, but it was felt that heretofore it had not been possible to induce cytogenetic changes by the application of reagents to embryonic cells in such an effective manner as was indicated by these preliminary studies. Researches by Nemec,[6] Winkler,[7] Jorgensen,[8] Randolph[9] and Kostoff,[3] had also shown that there were possibilities of inducing cytogenetic changes in various ways.

Blakeslee,[10,11] who made reference to the writers' unpublished results, and encouraged this publication has since proved beyond doubt that in *Datura*, tetraploids may be obtained from diploids after colchicine treatment. Likewise, Nebel[12] has been carrying out a series of cytological studies on both plant and animal tissue which indicated the same possibility. Thus it appears likely that the use of colchicine will prove to be of great practical importance to genetics.

Multinucleate structures were produced in *Allium* after colchicine treatment as shown in figures 9, 20, 21, 23, 25, 27, 28, 29, 30 and in *Zea mays* stem meristematic cells, figure 31. These conditions are probably due to prolonged treatment in solutions that were not entirely toxic. Each part of the multinucleate structure or compound nucleus represents the diploid number, the chromosomes of which are capable of dividing to produce an octoploid nucleus, figure 19. In this way octoploid nuclei are derived from tetraploid nuclei. It is possible for one part of the compound nucleus to divide, figure 9, while the other part remains in interphase. The result of this process would be a 48-chromosome or hexaploid nucleus, figure 6. The fact that polyploid nuclei have formed during treatment and that with prolonged treatment these polyploid nuclei again divided to form hexaploids

or octoploids, indicates the reason why colchicine is effective in polyploid induction. It is because this reagent does not inhibit chromosome division, but does prevent spindle and cell plate formation. The multinucleated structures are abnormal cytological monstrosities but may be regarded as components of polyploid nuclei in cells, some of which may still be capable of dividing later to form tissue. It is possible that this is the answer to the question regarding the peculiar effectiveness of colchicine as an agent.

The binucleate condition was observed in stem meristems of treated *Zea* tissue, figure 31. Another cell in *Zea* tissue showed that a portion of the nucleated component is capable of forming additional chromosomes, figure 22. The presence of conditions in stem meristems similar to root meristems indicated that principles of polyploid induction found to be true for roots were also true for stems.

The multinucleate portions adhere to each other so that separation of the compound nucleus from the cell does not break up the nucleus into its component nuclear parts. In many cells the polyploid condition is present in the form of a single nucleus. In these cases the existence of multiple numbers of chromosomes could be inferred by comparison of size of nucleus.

Induction of polyploidy by colchicine occurs in two general stages: namely (1) a stage marked by increased chromosome number without nuclear abnormalities and irregularities, and (2) the induction of polyploidy with abnormal shapes of nuclei and abnormal counts. The latter class is characteristic of cells treated for a long time and with greater concentrations of colchicine. These results were found in some cases recorded with abnormal nuclei, figures 18, 25, 27 and abnormal dividing chromosomes, figure 17. The former class marked by polyploidy without abnormalities was a group of changes produced by treatment for shorter periods of time and less concentrated solutions. It is that class of induced polyploidy which is capable of division during treatment and after treatment in which we are particularly interested, from the point of view of hereditary problems. The abnormal nuclei, figure 18, were only cytological monstrosities and more characteristic for the conditions produced by root tip treatments with chloral hydrate and other chemicals, or external environmental agents. Abnormal phases showed more fusions of chromosomes, fragments lost from nuclei and other more unusual cytological occurrences.

The physiological activity of the embryonic cell at the time of treatment is important because increase of cell activity means increase in number of mitotic divisions. This increase in mitotic division renders colchicine treatment more effective. The methods used in this study with treated seedlings already germinated and growing meristems will not yield precisely the same results when applied to the relatively inactive meristematic cells of dry seeds.

This study demonstrated that colchicine is effective in the production of cytogenetically changed cells. The process affects the mitotic divisions; hence, it is a study of independent cells as structural and functional units. These units divide mitotically at a given time and do so independently of the activity of the adjacent cells. The writer was not concerned with a study of entire tissues influenced by colchicine treatment. The exact rôle played by colchicine is essentially the inhibition of the mitotic spindle which prevents separation of the daughter nuclei, and cell plate formation, with the subsequent division into two cells. The failure of the reagent to interfere with the process of chromosome formation by longitudinal equational divisions, shows a specificity of a high degree for inhibition of certain phases of cell division and the apparent promotion of other phases of the mitotic process.

[1] Allen, E., *Anat. Rec. Abst.*, **67,** 49 (1936).

[2] Mr. E. L. Lahr gave the writer some helpful suggestions in the initiation of this investigation.

[3] Kostoff, D., *Genetica*, **12,** 33–118 (1930).

[4] Eigsti, O. J., *Manuscript Thesis*, University of Illinois (1935).

[5] Eigsti, O. J., *Bot. Gaz.*, **98,** 363–369 (1936).

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[7] Winkler, H., *Zeits. Bot.*, **8,** 417–531 (1916).

[8] Jorgenson, C. A., *Jour. Genet.*, **19,** 133–211 (1928).

[9] Randolph, L. F., these PROCEEDINGS, **18,** 222–229 (1932).

[10] Bakeslee, A. F., *Compt. Rend.*, **205,** 476 (1937).

[11] Blakeslee, A. F., *Jour. Hered.*, **28,** 392–411 (1937).

[12] Nebel, B. R., *Biol. Bull.*, **73,** 351–352 (1937).

CHEMICAL STRATIFICATION AND LAKE MORPHOLOGY

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Communicated January 11, 1938

Data collected during an extended investigation of Linsley Pond, a small eutropic lake (area 94,400 m.^{2}, max. depth 14.8 m., mean depth 6.7 m.) near North Branford, Connecticut, throw considerable light on the nature of the water-movements in the hypolimnia of thermally stratified lakes. That such movements occur is clear from the rise in concentration of substances, that can only have been derived from the bottom mud, at distances from the latter far exceeding those over which molecular diffusion could be effective. The nature of such water movements has been a matter of discussion, three main hypotheses having been advanced in recent years. Birge,[1] Thienemann[2] and more explicitly Grote,[3] have regarded

wind-generated turbulence as the most important agency in the transport of heat and dissolved material throughout the lake. This hypothesis implies that the hypolimnion is not really a closed system and that considerable vertical tubulent diffusion occurs. McEwen,[4] while admitting the fundamental importance of turbulence, believes that at least in the upper hypolimnion and in the epilimnion, a system of vertical convection currents generated by surface cooling, is of great importance. Alsterberg,[5] on the other hand, regards the hypolimnion as a closed system, in which water movements are confined to thin horizontal laminae, such movements having a negligible vertical component. This hypothesis appears to be the only one consistent with the remarkable optical microstratification discovered by Whitney.[6] It is perhaps unfortunate that in previous investigations most attention has been focused on temperature, in part controlled by direct radiation, and on oxygen, the most reactive of all dissolved substances in a lake, and in the present case known to be primarily controlled (Riley *unpublished*) by consumption and production in the free water. Evidence based on the distribution of alkalinity (bicarbonate ion, determined by titration with methyl orange as indicator) is given below in support of Alsterberg's hypothesis, though the results obtained on the oxygen deficit in Linsley Pond do not permit us to follow this author in all his conclusions.

Exclusion of Vertical Turbulent Transport.—In figure 1, the solid lines represent the variation, with depth, of alkalinity, the curves being based on the means of determinations made weekly, at one meter intervals, during three successive five-week periods, throughout the summer of 1937. It will be observed that during the period of investigation alkalinity tends to rise, but that the rise is much greater in the lower hypolimnion than in the epilimnion. Mineral analyses made at the end of September indicate that in the epilimnion sufficient calcium and magnesium exists to balance the bicarbonate, but that the concentration of these substances hardly varies with depth. In the hypolimnion, the excess bicarbonate is apparently balanced by considerable quantities of ammonia and iron, the latter presumably in the ferrous condition. This iron can only have come from the bottom and the large amounts of ammonia appear to indicate a like origin for the volatile alkali.

At any depth (y), the rate of change $\frac{\partial \theta}{\partial t}$ in any property (θ) with time due to turbulent diffusion is given by

$$\frac{\partial \theta}{\partial t} = \mu^2 \frac{\partial^2 \theta}{\partial y^2},$$

where (μ^2) is a virtual diffusion coefficient, the coefficient of turbulence, which is always positive. If the second derivative with respect to depth be negative, the first derivative with respect to time must be negative also.

If the three curves be compared, it appears that they show a marked similarity in form, showing points of inflection at the same depths. (Cf. also figure 2.) These points of inflection are such that in the lower water,

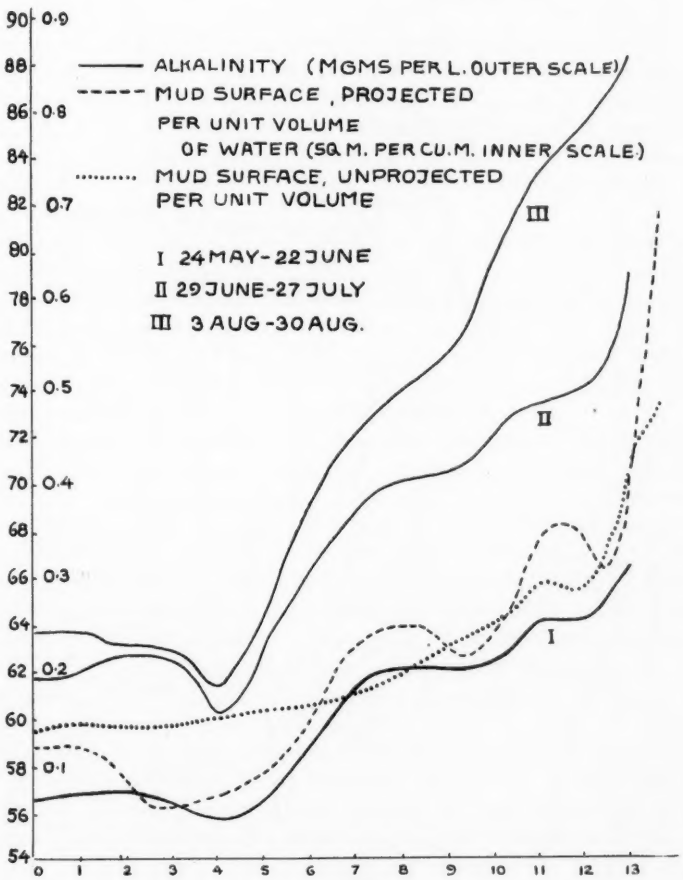

FIGURE 1

Solid lines, variation of alkalinity (HCO_3) with depth (mean values over successive five week periods). Broken line, ratio of projection of mud surface to volume. Dotted line, ratio of mud surface to volume.

the second derivative with respect to depth is persistently negative at 8 m. and 11 m. If the rise in alkalinity were due to turbulent movement bringing ferrous and ammonium bicarbonate up from a constantly re-

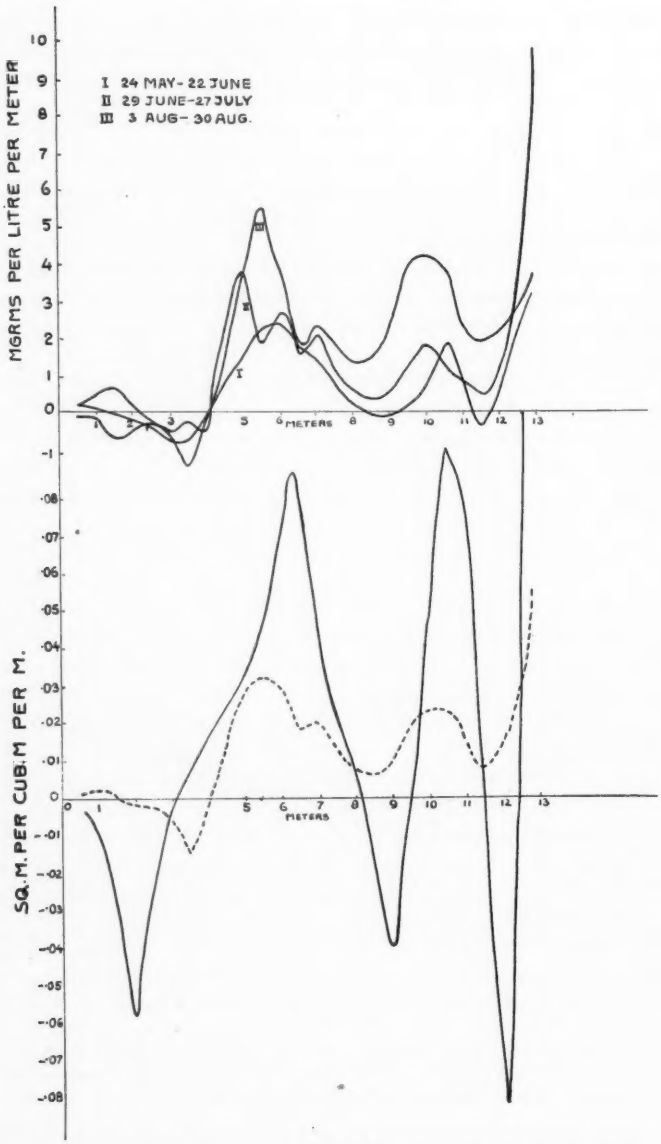

FIGURE 2

Upper curves, first derivative of alkalinity curves in figure 1. Lower curve, solid, first derivative of projected mud surface curve; broken, mean of the three upper curves.

generated and highly concentrated microzone on the mud, it is clear that the persistence of sections of the curve with a negative second derivative would be quite inconsistent with the continued accumulation of bicarbonate at all levels of the hypolimnion as a whole. A similar situation is apparent in the individual curves relating alkalinity to depth during periods of rapid rise in alkalinity. Such curves are less regular than the mean curves of figure 1, doubtless owing to uneven horizontal distribution at any given time, but regions with a negative second derivative can always be found, though not invariably at the same depths as in the smoothed curves. The unaveraged data, therefore, likewise give ample evidence of the occurence of considerable rises in alkalinity in layers of water where the turbulence hypothesis would, as in the case of the average curves, indicate that the alkalinity should be decreasing.

Particular significance is to be attached to the occurrence of a zone with a negative second derivative at 11 m., because, if McEwen's theory be accepted, this region should show the effects of almost pure turbulence uncomplicated by the type of convection current that this author has postulated. While it is hoped in the future to examine the implications of this very complex theory in greater detail, the correlation indicated below strongly suggests that it is inapplicable in the present case.

Morphological Concomitants of Horizontal Streaming.—As Alsterberg himself pointed out, if the hypolimnion be regarded as closed, and if its water movements are practically exclusively horizontal, a relationship should exist between the chemical characters of any layer of free water, and the amount of mud surface to which the edges of the layer are exposed. In attempting such a correlation it must be remembered that a steeply sloping lake bottom will probably neither receive nor retain as much mud as one that is more horizontal. A practical investigation of this problem would be extremely difficult, but as a first approximation it has been assumed that the chemically active mud is proportional not to the actual area of the lake bottom, but to this area projected on to a horizontal plane.

The broken line in figure 1 represents a curve drawn by plotting at 0.5 m., 1.5 m. and at succeeding meter intervals, the ratio of the area between successive one meter contours on a bathymetric map of the lake, to the volume enclosed between the planes represented by these contours. A comparison of this curve with the alkalinity curves of the same figure indicates that a relationship of the type demanded by Alsterberg's horizontal current hypotheses does exist. This is further brought out when the derived curves in figure 2 are examined. Owing to the large amount of steep lake bottom, particularly on the western side of the basin, the relationship is obscured when the ratio of contour length to area is taken as the measure of mud to water at any depth (dotted line in figure 1). The existence of any such relationship, even though it involve an empirical

but quite objective correction, speaks very strongly in favor of the hypothesis of horizontal movement, and this, taken in conjunction with the

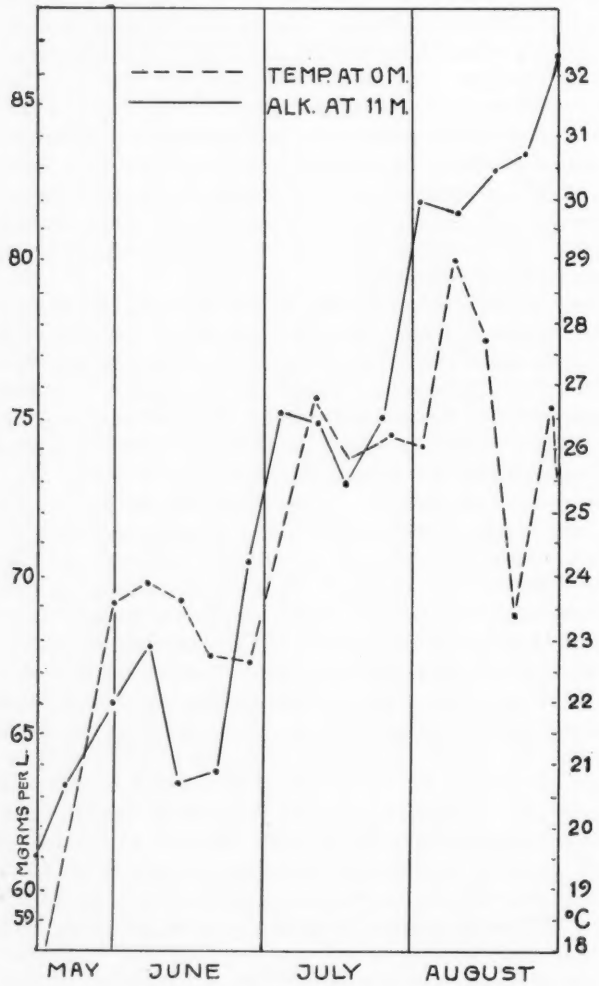

FIGURE 3

Variation of alkalinity at 11 m. (solid), and of temperature at 0 m. (broken), with time.

evidence given above, makes any other hypothesis of hypolimnetic water movements most improbable.

Temporal Variation in Alkalinity.—Conclusions based on comparison of the details of curves showing the variation of alkalinity with time at any given depth, are, for various technical and other reasons, less satisfactory than those based on the depth distribution curves for any given day. Although the significance of certain features in such curves is doubtful, the solid line in figure 3, representing the alkalinity at 11 m. from the end of May to the end of August, and typical of the events of all depths, indicates marked discontinuities in the rise of alkalinity during this period. The cause of the minima before the sudden rises is at present obscure, the two large rises (22 June–6 July and 27 July–3 August) certainly lie outside experimental error, and since the high values reached are in each case maintained almost unchanged for a week, they must be regarded as real and general throughout the water layer. These two large rises, and a third discontinuous rise beginning on the 24 August, appear to be initiated by temperature minima *at the surface* (broken line). Examination of the density curves at the time of such minima indicated that the stability of the top two meters of the lake is reduced at such times and rises more suddenly between two and three meters than at times of temperature maxima. This suggests that though the rise in alkalinity cannot be due to vertical turbulent diffusion, the best conditions for such a rise are provided by the existence of a well defined and freely circulating epilimnion of measurable thickness below which the stability rises sharply. Preliminary experiments with small scale models indicate that horizontal streaming of the type postulated can be produced in a lower, more dense layer when a less dense layer of water lies upon it, and when the boundary between the two is made to oscillate by an air current which causes turbulent motion in the upper layer, but no mixing of the upper, less dense water with the denser water at the bottom of the tank. It is hoped to be able to investigate the nature of such currents in models under more favorable conditions in the near future.

Intensive work in 1937 was made possible by a grant from the BACHE FUND, of the NATIONAL ACADEMY OF SCIENCES, here gratefully acknowledged. My thanks are also due to Professor A. E. Parr, for the loan of apparatus, to Mr. H. J. Turner and Miss Anne Wollack for the care they have taken over analyses entrusted to them, to Dr. G. A. Riley for unpublished morphometric data and to Dr. Riley and Mr. E. S. Deevey for help in the field.

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ASCORBIC ACID AND THE GROWTH OF PLANT EMBRYOS

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Introduction.—In an earlier paper it has been shown[1] that excised pea embryos of the variety "Perfection" may be satisfactorily grown upon sterile nutrient medium, particularly if various "accessory growth factors" of known chemical composition are provided. It was shown that there are at least four of these growth factors, one of which is ascorbic acid. It is ascorbic acid with which the present communication is particularly concerned.

There have been, previously, conflicting reports as to the effect of ascorbic acid upon plant growth. Havas,[2] von Hausen[3] and Davis, Atkins and Hudson,[4] among others, have found that ascorbic acid may exert a beneficial effect upon the growth of certain plants. It is of particular importance in the present connection, however, that Kögl and Haagen-Smit,[5] who grew pea embryos *in vitro* under conditions similar to those used by the present authors, failed to find any growth factor activity for ascorbic acid. They worked with pea varieties other than "Perfection," and a varietal difference in the ability of pea embryos to cover their ascorbic acid requirements by synthesis might therefore be suspected. It will be shown below that the embryos of different pea varieties do indeed differ widely in their ability to synthesize ascorbic acid from sucrose. It will in addition be shown that those varieties which synthesize the smallest amounts of this substance are those which respond (with increased growth) to its addition to the culture medium. The varieties which synthesize relatively large amounts of ascorbic acid, on the other hand, apparently furnish themselves with sufficient amounts of the substance and do not respond to its addition to the external medium.

Methods.—The nutrient medium used for the culture of the excised embryos was that used in the previous experiments,[1] which contained, per liter, the following constituents: $Ca(NO_3)_2 \cdot 4H_2O$, 236 mgs.—$MgSO_4 \cdot 7H_2O$, 36 mgs.—KNO_3, 81 mgs.—KCl, 65 mgs.—KH_2PO_4, 12 mgs.—ferric tartrate, 1.5 mgs.—sucrose, 40 gms. and agar, 10 gms. Fifteen cc. of this medium was placed in each of the 50 cc. Erlenmeyer flasks which were used as the culture vessels. 0.75 Mgs. of ascorbic acid was added to each culture flask in the ascorbic acid cultures. The pea seeds were sterilized in 95% alcohol and 0.1% $HgCl_2$, and were then soaked in sterile water for six hours prior to excision of the embryos. The length of time for which the seeds are soaked is of considerable importance, since with times longer than six hours, increasing amounts of the various accessory growth factors are ap-

parently mobilized from the cotyledons by the embryo. Twenty embryos were used for each treatment or variety in each experiment, and each experiment consisted of 10 parallel portions, or a total of 200 embryos. The actual culturing was carried out in a special culture room, after which the embryos were grown in a dark room thermostatically controlled at 24 C. Ascorbic acid determinations were made by extraction of the tissues with 2% meta-phosphoric acid in 8% acetic acid according to the method of Muslin and King,[6] and subsequent titration of the extract with a standard 2-6-dichlorphenolindophenol solution in a micro-burette.

Growth measurements of the seedling shoots were made weekly for four weeks from the time of excision of the embryos, and the measurements from

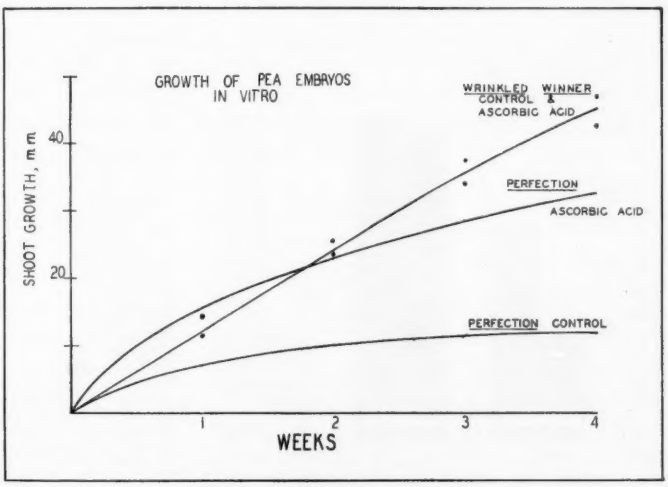

FIGURE 1

The growth rate of pea embryos *in vitro*, the varieties "Perfection" and "Wrinkled Winner," each with and without the addition of ascorbic acid to the nutrient medium.

a typical experiment are presented in figure 1. Preliminary experiments also showed that the ascorbic acid content rises to a maximum within two weeks and that it remains at approximately this maximum level for the following two weeks. The ascorbic acid determinations were therefore made only on the plants whose growth had been followed for four weeks. This had of course the advantage that the growth measurements and the ascorbic acid determinations were made upon the same individual plants. In the discussion below only the shoots will be considered. It was, however, found that there is a close correlation between the ascorbic acid content of root and shoot.

Experimental Results.—Nine varieties of pea embryos were compared as to synthesis of and growth response to ascorbic acid.[7] The length of shoot, response to ascorbic acid and content of ascorbic acid, all at the expiration of four weeks, are summarized for each of these nine varieties in table 1 and presented graphically for two typical varieties in figure 2. It may be seen at once that the varieties fall sharply into two classes, i.e., those which respond to added ascorbic acid with greatly increased growth, and those which show little or no growth response. Of the latter the variety

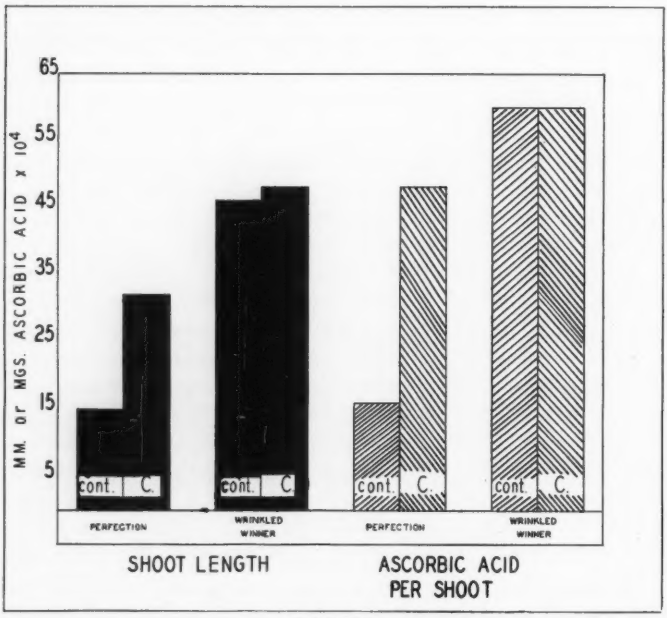

FIGURE 2

The growth and the ascorbic acid content of pea embryos, varieties "Perfection" and "Wrinkled Winner" after four weeks *in vitro*. "cont." = control cultures, without added ascorbic acid. "C." = cultures receiving ascorbic acid.

"Wrinkled Winner" is the best example, since the four other varieties proved more difficult to grow *in vitro* and gave rather irregular results. Correlated with growth response to added ascorbic acid is *low ascorbic acid content* of the variety when it is grown on the vitamin free basic medium. The varieties "Perfection" and "Alaska" which respond vigorously contain less than one-third as much ascorbic acid as embryos of "Wrinkled Winner" which does not respond. Neither "Perfection" nor "Wrinkled

Winner" embryos contain appreciable amounts of ascorbic acid when they are excised from the seed at the end of six hours' soaking. The above differences of ascorbic acid content of the seedlings must then be due to *differences in the ability of the various varieties to synthesize ascorbic acid.* We see at once that the fact that some varieties of pea embryos do not respond to ascorbic acid does not by any means justify the conclusion that such embryos do not need this substance. It would rather seem to indicate that such embryos are capable of synthesis of ascorbic acid in quantities sufficient to fulfill their requirements.

Plants which are supplied with ascorbic acid actually take it up and their content of this substance is correspondingly increased above that of control plants, as is also shown in table 1 and graphically in figure 2. The ascorbic acid content of the variety "Perfection" when supplied with ascorbic acid, is not greatly below that of the variety "Wrinkled Winner" which synthesizes the substance in adequate amounts. Thus, when "Perfection" embryos are supplied with ascorbic acid, their vitamin content as well as their growth rate increases to a level comparable to that of the ascorbic acid "auto-trophic" variety, "Wrinkled Winner."

TABLE 1

ASCORBIC ACID CONTENT AND GROWTH RESPONSES OF NINE VARIETIES OF PEA EMBRYOS *in Vitro*

VARIETY	TOTAL PLANTS USED	AVERAGE GROWTH IN 4 WEEKS; MM.			MGS. ASCORBIC ACID PER; GM. WET WEIGHT, (CONTROL)		
		CONTROL	ASCORBIC ACID	(% OF CONTROL)	CONTROL SHOOT	ASCORBIC SHOOT	
Perfection	100	15	32	213	0.0016	0.0048	0.064
Alaska	40	23	46	200	0.0016	0.0035	0.075
Daisy	40	10	17	170	0.0015	....	0.050
British Empire	40	11	18	163	0.0012	....	0.054
Delacatesse	20	29	33	114	0.004	....	0.138
Little Marvel	20	37	38	103	0.006	....	0.120
Laxtons Progress	20	24	25	104	0.006	....	0.167
Stratagem	20	22	23	104	0.005	....	0.145
Wrinkled Winner	100	46	48	104	0.006	0.006	0.172

The control embryos of the five varieties which synthesize relatively large amounts of ascorbic acid also grow somewhat better than the control embryos of the four varieties which are ascorbic acid "hetero-trophic," although the variety "Alaska" is apparently an exception. It seems nevertheless that in general, ability to synthesize ascorbic acid is correlated not only with a lack of response to added vitamin, but also with a larger absolute growth in the absence of added vitamin.

As mentioned above, the excised pea embryo is able to respond with increased growth to several different accessory factors, even though one of these factors be added to the medium in the absence of the others. Thus, aneurin (vitamin B_1) alone acts as an accessory growth factor just as does

ascorbic acid.[1] However, the addition of aneurin to the medium considerably increases the amount of ascorbic acid present in the plant, as is

TABLE 2

INFLUENCE OF ANEURIN (VITAMIN B_1) ON THE ASCORBIC ACID CONTENT OF "PERFECTION" PEA EMBRYOS

	GROWTH IN 4 WEEKS; MM.	ASCORBIC ACID CONTENT; MGS. PER SHOOT
Control Embryos	15	0.0018
Aneurin Embryos	40	0.0052

shown in table 2. In this typical experiment there was found to be nearly three times as much ascorbic acid in the plants which received aneurin (0.01 mgs. per culture flask[8]) as in those which did not. The nature of this effect cannot as yet be explained, but it is of interest to note that Svirbely[9] reports that synthesis of ascorbic acid by the rat depends upon an adequate supply of vitamin B_1.

Discussion.—The point which it is particularly desired to emphasize in the present paper is that an organism will or will not respond to a given accessory growth factor depending, among other things, on (*a*) the need of the organism for the factor in question, and (*b*) the ability of the organism to synthesize the substance. The fact that a given organism does not respond to a given growth factor may then mean only that the particular organism is equipped to synthesize it in adequate amounts; it does not necessarily mean, however, that the factor in question plays no rôle in the economy of the organism. This principle has long been familar to the animal physiologists, who realized that the rat, for example, covers its ascorbic acid requirements by synthesis. The same principle has also been demonstrated for the yeasts by Lucas[10] and others, and more recently for other of the fungi by Kögl and Fries.[11] It would seem important that this principle be also recognized in plant physiology, in order that controversies as to the "essential" or "non-essential" nature of the various growth factors be avoided. It has been shown above that plants as closely related as the various varieties of peas differ greatly as to their response to ascorbic acid. For varieties such as "Perfection" and "Alaska" ascorbic acid would seem, superficially, to be more nearly an essential growth factor than for varieties such as "Wrinkled Winner." In reality, however, these varieties differ rather in their ascorbic acid auto-trophism, in their ability to synthesize the substance, and it would seem justifiable to conclude that ascorbic acid is quite as much a growth factor for "Wrinkled Winner" as for "Perfection."[12]

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[7] For seed of the various pea varieties the authors are greatly indebted to the Ferry-Morse Seed Co., San Francisco, and to the Calif. Packing Corp., San Francisco.

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POTENTIALS IN HALICYSTIS AS AFFECTED BY NON-ELECTROLYTES

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In diluting the sea water surrounding marine algae it is desirable to add something to maintain the osmotic pressure, otherwise the cells take up water and may burst. Damon[1] used glycerol for this purpose in experiments on *Valonia*. He found that the changes in P.D. at 20°C. obeyed the equation

$$\text{P.D.} = 58\,\frac{V_{Cl} - U_{Na}}{V_{Cl} + U_{Na}}\log\frac{C_1}{C_2}$$

where V_{Cl} and U_{Na} are the apparent mobilities of Cl^- and Na^+ in the outer protoplasmic surface, C_1 is the higher and C_2 the lower concentration of sea water. Putting $C_{Cl} = 1$ he obtained 0.2 for the value of U_{Na} (on the assumption that $C_1 \div C_2$ was the same in the protoplasmic surface as in the external solution).

The limiting value of the P.D. in this case may be obtained by putting $U_{Na} = 0$. We then obtain 17.4 mv. as the limit when $C_1 \div C_2 = 2$. This value is a little less when we employ activities in place of concentrations.[2]

Similar experiments with *Halicystis*[3] yield a different result. In view of the work of Blinks[4] it was decided to add sufficient $CaCl_2$ and KCl to the glycerol solution to make the concentration approximately the same as in sea water. The solution contained 1.1 M glycerol + 0.02 M $CaCl_2$ + 0.012 M KCl.

When sea water is diluted with an equal volume of this solution (at pH 8.2) and is then substituted for natural sea water (at pH 8.2) we find that in

one minute[5] or less the P.D. becomes about 29 mv. less positive.[6] Since this value is in excess of the theoretical limit of 17.4 mv. it seems possible that glycerol has altered the value of $C_1 \div C_2$ in the protoplasmic surface so that instead of being 2, as assumed, it has reached some higher value.

This might happen as follows. If the partition coefficient S (conc. of electrolyte in protoplasmic surface $\div$ conc. of electrolyte in external solution) were constant, the value of $C_1 \div C_2$ would be the same as in the external solutions. But if S in the dilute sea water is lessened by the glycerol, the value of C_2 will be correspondingly small and that of $C_1 \div C_2$ will be greater.

It seems advisable, for the present at least, to employ the term partition coefficient in a very broad sense since, for example, the effect of NaCl may depend not only on the number of sodium ions in the protoplasmic surface but also on complexes in the sense of Kraus,[7] e.g. $(NaX_I)^+$ and $(NaX_{II})^+$ where X is an element or a radical. All such complexes in the protoplasmic surface plus the actual sodium ions may be lumped together and their sum in unit volume of the non-aqueous surface layer divided by the sum of the potassium ions in unit volume of the external solution may be called the "apparent partition coefficient."

A variety of other explanations is possible, such as the following:

(1) Mechanical rupture of the outer protoplasmic surface layer, the process being fully reversible. If this layer is liquid, as seems possible, this process might take place as in (unpublished) experiments with oily films spread out on the surface of aqueous solutions when chemical action is going on. In such cases breaks in the film may appear and disappear as the result of local action. A great many are usually present and the amount of short-circuiting and consequent loss of P.D. might change gradually, depending on the number of breaks in the film.

This process might cause a total loss of P.D. at either protoplasmic surface. If it occurred at one surface while the other had a negative P.D. the resultant P.D.[8] would be negative.

(2) Production of organic ions in the protoplasm which lessen the outwardly directed potential.

(3) Changes in potentials not due to diffusion, e.g., in phase boundary potential or in membrane potential.[9]

Glucose, sucrose and maltose act like glycerol, as shown in table 1. One striking effect is common to all of these, namely, the recovery of the original P.D. on standing .[10] For example, when sea water is replaced by sea water plus an equal volume of isotonic glycerol solution (containing $CaCl_2$ and KCl) the P.D. becomes less positive in the course of about 20 seconds to the extent of about 29 mv. But a few seconds later the P.D. begins to grow more positive again[11] as though the non-electrolyte were penetrating to the

inner protoplasmic surface and there setting up changes opposite in sign to those produced at the outer surface. Such changes at the two surfaces might very well be opposite in sign since the surfaces are known to differ greatly.[12] They would be possible even if the surfaces were alike provided ions produced in the protoplasm diffused inward as well as outward.

TABLE 1

CHANGE OF P.D. PRODUCED BY REPLACING SEA WATER IN CONTACT WITH *Halicystis* BY SEA WATER PLUS AN EQUAL VOLUME OF AN ISOTONIC SOLUTION CONTAINING 1.1 M NON-ELECTROLYTE + 0.02 M $CaCl_2$ + 0.012 M KCl.* ALL AT pH 8.2 UNLESS OTHERWISE STATED

NON-ELECTROLYTE IN SUBSTITUTED SOLUTION	CHANGE IN P.D.	NUMBER OF OBSERVATIONS†
Glycerol	28.9 ± 0.83	5
Glucose	27.8 ± 4.74	6
Sucrose	17.1 ± 1.56	6
Maltose	38.0 ± 1.59	13
Mannite	13.8 ± 0.52	14
Mannite pH 6.4	9.8 ± 0.68	13

* In each case the P.D. became less positive (the sign is positive when the positive current tends to flow outward from the sap to the external solution).

† A limited number of cells was available.

In the course of 5 minutes or less the P.D. usually returns approximately to the original value in sea water. If the cell is then transferred to sea water little or no change in P.D. occurs.[13] This may mean that the glycerol is washed out of both surfaces at about the same rate so that the changes in P.D. at one surface are cancelled by those at the other.

A different picture is presented when we employ mannite.

(1) With mannite the theoretical limit is not as a rule exceeded. The average loss of P.D. when sea water is replaced by sea water plus an equal volume of 1.1 M mannite + 0.02 M $CaCl_2$ + 0.012 M KCl at pH 8.2 is about 14 mv. An occasional measurement runs higher[14] (up to 22 mv.). Experiments at pH 6.4 gave a lower value (table 1). These were made by lowering the pH of the sea water from 8.2 to 6.4 and then transferring to the dilute sea water at 6.4. The low value may be connected with the fact that the P.D. is reduced by lowering the pH of the sea water (this will be discussed in a subsequent paper).

(2) The P.D. thus produced remains constant for 8 minutes or more. It does not as a rule increase again and show "recovery."[15]

In view of this it would seem advisable to use mannite in studying the concentration effect. We cannot be sure that it does not affect the values obtained but it appears to do so less than the other substances mentioned.

The high value obtained for the concentration effect with mannite is of interest. If we use this value to calculate $U_{Na} \div V_{Cl}$ we obtain a value very much less than that found for *Valonia*[1] (in *Valonia* $U_{Na} \div V_{Cl} = 0.2$).

To account for the values in table 1 in excess of the theoretical, the simplest assumption seems to be that partition coefficients are altered by all the non-electrolytes except mannite. Unfortunately the theory[16] of partition coefficients is not yet developed and at present we can do little more than record suggestive facts. If non-electrolytes can alter the partition coefficients of electrolytes it is evident that this may be of importance for the cell.

It would seem that if glycerol lessens the partition coefficients of electrolytes, as suggested, the addition of glycerol to sea water should make the P.D. less positive. This is the case. Enough glycerol was added to sea water to increase the osmotic pressure by about 50 per cent. Enough solid NaCl was then added to bring the halide content up to the normal (0.58 M). In the course of 3 to 5 minutes the P.D. became less positive by 10 to 20 mv. On replacing in sea water the P.D. returned to normal in 4 minutes or less, but in the meantime it became temporarily still less positive to the extent of 5 to 10 mv.

Enough mannite was added to sea water to raise its osmotic pressure about 50 per cent and solid NaCl was then added to make the halide content 0.58 M as usual. Since this produced no change in P.D. we might conclude that, as expected, mannite does not change the partition coefficient as glycerol does. But mannite is not wholly without effect on the P.D. for when the cells are replaced in sea water the P.D. becomes temporarily less positive to the extent of 10 to 15 mv. After this it returns to normal (the whole process takes about 4 minutes). The cause of these changes is presumably osmotic and is due to the taking up of water by the different parts of the protoplasm. As might be expected this is similar with glycerol and with mannite.

That the taking up of water can cause the P.D. to become less positive is suggested by experiments with sea water plus an equal volume of distilled water. In this the P.D. became within 15 seconds 44 ± 3 mv. less positive (10 observations).[17] Similar experiments performed by L. R. Blinks yielded smaller values.[18]

Replaced in sea water the cells returned in 4 minutes or less to the normal P.D.[19] In this case we appear to have an effect due to change of electrolyte concentration plus an effect due to the redistribution of water. The effect is therefore much greater than that produced by mannite.

Summary.—To study changes in P.D. caused by diluting the sea water bathing cells of *Halicystis* it is desirable to add a non-electrolyte to maintain the osmotic pressure of the external solution. For this purpose mannite appears to be one of the most suitable. With glycerol, glucose, sucrose and maltose the changes of P.D. are so large that a reversible alteration of the protoplasmic surface is indicated. This may affect the P.D. by changing the partition coefficients of electrolytes or in other ways.

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[2] Osterhout, W. J. V., *Ibid.*, **13,** 715 (1929–30).

[3] The experiments were done with *Halicystis Osterhoutii* (Blinks, L. R., and Blinks, A. H., *Bull. Torrey Bot. Club*, **57,** 389 (1931)), using the technique described in a previous paper (Osterhout, W. J. V., *Jour. Gen. Physiol.*, **20,** 13 (1936–37)). Temperature about 22°C.

Unless otherwise stated there was no appearance of injury during the treatment or in the following days.

[4] Blinks, L. R., *Jour. Gen. Physiol.*, **13,** 223 (1929–30); **18,** 409 (1934–35).

[5] There is a latent period of 15 seconds or less. This is very variable and may depend to some extent on the thickness and cutinization of the cellulose wall and on bacterial jelly covering the cell. Such jelly gives the cells a slippery feeling. There seemed to be no jelly on these cells.

This latent period was also observed in experiments with KCl, NH_4Cl at pH 8.2 (0.005 M but not with 0.3 M), and 0.01 M guaiacol.

[6] See table 1. A similar result was previously obtained by L. R. Blinks (personal communication).

[7] Cf. Osterhout, W. J. V., *Jour. Gen. Physiol.*, **20,** 13 (1936–37).

[8] For reversal of sign of *Halicystis ovalis* in unbalanced NaCl see Blinks, L. R., Rhodes, R. D., and McCallum, G. A., *Proc. Nat. Acad. Sci.*, **21,** 123 (1935). For reversal in *Valonia* caused by dilute sea water see Damon, E. B., and Osterhout, W. J. V., *Jour. Gen. Physiol.*, **13,** 457 (1929–30).

[9] Cf. Teorell, T., *Proc. Soc. Exp. Biol. Med.*, **33,** 282 (1935), Meyer, K., and Sievers, J.-F., *Helv. Chim. Acta*, **19,** 987 (1936).

[10] This has been observed with glycerol by L. R. Blinks (personal communication).

[11] This does not happen in *Valonia.*

[12] Cf. Blinks, L. R., *Jour. Gen. Physiol.*, **13,** 223 (1929–30); **18,** 409 (1934–35).

[13] If the cell is transferred to sea water when the P.D. is at the minimum (i.e., before "recovery" has started) the P.D. becomes temporarily less positive to the extent of 4 mv. or less; this does not last more than a minute and the P.D. then returns to the normal value. This also applies to mannite. Apparently it does not apply to glucose. Presumably this means a different behavior at the inner and outer protoplasmic surfaces.

[14] Blinks found that a lack of balance in the external solution made the P.D. less positive or even strongly negative. This may play a rôle here.

Cells injured by exposure to a temperature below 14°C. showed no change in P.D. when exposed to sea water plus an equal volume of mannite solution but when glycerol was used in place of mannite they showed the expected change. In both cases the cells were dead the next day. A few lots of cells showed no change in P.D. when transferred to sea water plus an equal volume of mannite solution even though they seemed normal in every other respect. One such lot when tested two days later gave the usual response.

[15] An occasional cell shows a small decrease. Such cells become temporarily less positive when returned to sea water, as in the case of cells exposed to glycerol, but this temporary change is much less than with glycerol.

[16] Cf. Shedlovsky, T., and Uhlig, H. H., *Jour. Gen. Physiol.*, **17,** 549, 563 (1933–34); Falkenhagen, H., *Electrolytes*, Oxford, Clarendon Press, 1934.

[17] If left in the solution 30 seconds or more the P.D. began to increase (as with glycerol) but in most cases the cell was returned to sea water after 20 seconds.

[18] Personal communication.

[19] After the cell was replaced in sea water the P.D. did not become temporarily less positive as in the case of mannite and glycerol.

A COMPARISON OF THE DIFFUSIBLE SUBSTANCES CONCERNED WITH EYE COLOR DEVELOPMENT IN DROSOPHILA, EPHESTIA AND HABROBRACON*

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Diffusible substances have been postulated as being concerned in eye color development in insects belonging to three orders, Ephestia[1] and Bombyx[2] (Lepidoptera), Drosophila[3] (Diptera) and Habrobracon[4] (Hymenoptera). It is known that the substances concerned in Ephestia and Drosophila are not species-specific.[5,6] It is a matter of some interest to determine the relation to one another of the hormones or hormone-like substances in these insects. For example, is the "A-hormone" of Ephestia identical with either "v^+ substance" or cn^+ substance of Drosophila?[7] In connection with this example, Ephrussi[8] has given a comparative summary of the information concerning the A-hormone of Ephestia and the diffusible substances of Drosophila. In the present communication tests of Ephestia for substances active in Drosophila are reported; other experiments planned to show the relation between substances present in Drosophila and those in Habrobracon are described.

Material and Methods.—In the tests of Ephestia, wild type larvae, regularly used as hosts in culturing the parasitic wasp Habrobracon, were used. In making tests in Drosophila, vermilion brown (*v bw*) animals were used as a test for v^+ substance and cinnabar brown (*cn bw*) animals as a test for cn^+ substance. Wild type Drosophila pupae were used as a source of v^+ and cn^+ substances. Cinnabar pupae were similarly used as a source of v^+ substance alone. In tests of Habrobracon, six eye color types were used: wild, orange (*o*), ivory (o^i), red (*rd*), cantaloup (*c*) and white (*wh*). We are grateful to Doctor P. W. Whiting, who kindly placed these stocks of Habrobracon, as well as the material of Ephestia, at our disposal.

In all cases in which Habrobracon was used as a source of extracts (table 1) these extracts were made by heating larvae and pupae, taken shortly after cocoon formation, to 100°C. for a half minute or so, crushing and then removing the fluid fraction by centrifuging through a glass wool and asbestos filter.[9] Such extracts were tested by injection into Drosophila test larvae in amounts of approximately 0.5 to 1.0 cubic mm. per larva. In certain tests, designated in table 1, a feeding technique was used.[10] From 25 to 75 larvae and pupae (used shortly after spinning of cocoons) were heated to 100°C. for about 40 seconds, crushed and fed to 10 to 12 test larvae of Drosophila some 60 to 72 hours after egg laying (25°C.). The extract

of cinnabar Drosophila pupae was prepared in essentially the same way as described above for Habrobracon. The extracts of wild type pupae injected into ivory Habrobracon were prepared in a more elaborate way by Doctor Edward L. Tatum, to whom we are grateful for making them available.

TABLE 1

MODIFICATIONS OF THE EYE COLOR OF DROSOPHILA BY DIFFUSIBLE SUBSTANCES FROM HABROBRACON AND THE RECIPROCAL

SOURCE OF SUBSTANCES	RECIPIENT	METHOD OF TREATMENT	NUMBER OF SEPARATE TESTS	NUMBER OF TEST ANIMALS POSITIVE	NUMBER OF TEST ANIMALS NEGATIVE	STRENGTH OF EFFECT
Wild Hab.	*v bw* Dros.	Extract injected	3	13	1	1.1
Same	Same	Fed	2	16	0	1.8
Wild Hab.	*cn bw* Dros.	Extract injected	3	14	1	1.0
Same	Same	Fed	3	23	0	2.0
Orange Hab.	*v bw* Dros.	Extract injected	2	9	0	1.6
Same	Same	Fed	2	17	0	1.9
Orange Hab.	*cn bw* Dros.	Extract injected	2	0	9	0.0
Same	Same	Fed	2	0	16	0.0
Ivory Hab.	*v bw* Dros.	Extract injected	1	14	0	2.3
Same	Same	Fed	2	18	0	2.5
Ivory Hab.	*cn bw* Dros.	Extract injected	1	0	16	0.0
Same	Same	Fed	2	0	18	0.0
Red Hab.	*v bw* Dros.	Extract injected	1	8	0	3.0
Same	Same	Fed	1	11	0	2.2
Red Hab.	*cn bw* Dros.	Extract injected	1	7*	0	0.1
Same	Same	Fed	1	10	0	2.1
White Hab.	*v bw* Dros.	Extract injected	1	5	0	1.0
Same	Same	Fed	2	16	0	2.0
White Hab.	*cn bw* Dros.	Extract injected	1	7*	0	0.2
Same	Same	Fed	2	9*	13*	0.2
Cantaloup Hab.	*v bw* Dros.	Fed	1	8	0	3.1
Cantaloup Hab.	*cn bw* Dros.	Fed	1	9	0	2.8
Wild Dros.	Ivory Hab.	Purified extract injected	2	32	2	brown
Cinnabar Dros.	Ivory Hab.	Extract injected	1	0	7	none
Cinnabar Dros.	*v bw* Dros.	Extract injected	1	12	0	1.0
	(Control for above experiment, same extract used)					
Ringer's solution	Ivory Hab.	Injected (operation control)	1	0	6	none

* Classification as "positive" or "negative" not certain.

Strengths of tests are recorded according to an arbitrary numerical scale, 0 representing no modification and 5 the maximum change possible. It

should be emphasized that the numerical values obtained in different tests are not strictly comparable; to be so, tests must be run in parallel and the test animals directly cross-compared.

Tests of Ephestia.—One apparent difference between the A-hormone of Ephestia and either v^+ or cn^+ substance of Drosophila is the fact that A-hormone is produced and released by gonad transplants[1] whereas neither v^+ nor cn^+ substance appears to be released by such transplants in Drosophila.[11] Extracts were made of larval testes of Ephestia by heating the excised testes in Ringer's solution. Such extracts gave a positive test for both v^+ substance (21 test animals, all positive, average color value 1.5) and cn^+ substance (13 test animals, all positive, average color value 1.7). Similar tests of larval testes of Drosophila are negative for both substances (15 and 10 individuals in tests for v^+ and cn^+ substances). Transplanted testes of wild type Drosophila are likewise without effect on the eye color of either vermilion brown or cinnabar brown hosts (11 and 10 individuals with well developed implants in the respective two tests). It is thus clear that extracts of Ephestia testes contain substances similar in effects to those found in Drosophila. These facts, in connection with the general similarity in effects of A-hormone and the Drosophila substances and the similarities in solubility properties,[9,12,13] suggest that the A-hormone might well be identical with either v^+ or cn^+ substance. Appropriate tests of the red eyed mutant of Ephestia, not available to the authors, should show with which, if either, of the Drosophila substances the A-hormone might be identical.

Tests Involving Habrobracon.—It has been shown that in certain types of double nucleus mosaics in Habrobracon the eye color characters orange and ivory (differentiated by the genes o and o^i, members of an allelic series) are non-autonomous.[3] There appears to be a diffusion of some substance from orange-plus (o^+) to orange (o) or ivory (o^i) tissue such that orange or ivory eye tissue is modified in the direction of wild type (black). If this assumed substance is identical with one or the other of the eye color substances known in Drosophila, it should be possible to demonstrate the presence of the substance in wild type Habrobracon by tests made on Drosophila. It should likewise be possible to demonstrate that the substance concerned is either absent or reduced in amount in orange or ivory Habrobracon.

Such tests have been made following the methods outlined in a previous section of this paper. The results are summarized in table 1. Extracts of wild type wasps are positive for both substances. Both orange and ivory wasps gave extracts positive for v^+ substance but negative for cn^+ substance. These results suggest that the substance concerned in the differentiation of orange and ivory from wild type is similar to, or identical with, cn^+ substance of Drosophila. Reciprocal tests, extracts of Drosophila

pupae injected into Habrobracon larvae or pupae, show that wild type Drosophila pupae contain a substance capable of modifying ivory eye color toward wild type. Furthermore, an extract of cinnabar pupae, known from previous experience[9] to contain v^+ substance but not cn^+ substance, was without apparent effect on the eye color of ivory Habrobracon pupae. A parallel test of the same extract of cinnabar pupae was made in vermilion brown larvae to determine whether v^+ substance was actually present in the particular extract used in Habrobracon; the test was positive.

Although the proof cannot be considered to be complete, the above facts do provide a reasonable basis for postulating that the substance deficient in orange or ivory wasps is the same as that deficient in cinnabar flies.

Three additional eye color types of Habrobracon have been tested for both v^+ and cn^+ substances. Red and cantaloup give tests for both substances as strong or stronger than wild type (table 1). Assuming that the indicated differences in the amount of substances are significant, it is suggested that the relation between pigment formation and utilization of diffusible substances found by Ephrussi and Chevais[14] in Drosophila might account for them. Extracts of white Habrobracon larvae and pupae give positive tests for v^+ substances but are very low in activity in tests for cn^+ substance. In fact, cn^+ substance tests of white wasps are so weak that it is only by the most careful comparison with control test animals that a modification can be detected. On the basis of these tests alone, one would hesitate to differentiate between white and ivory. However, it is known from studies of mosaics that the white character is autonomous in development and in addition that white tissue in such mosaics is capable of producing and releasing a substance that modifies orange eye tissue in the same apparent manner as does cn^+ substance.[4]

Discussion.—The host-parasite relations between Ephestia and Habrobracon are of interest in relation to the eye color substances. Ephestia larvae contain both v^+ and cn^+ substances. It is known that both of these substances can be effectively administered to Drosophila by feeding.[10] Furthermore, it has been shown that cn^+ substance (from Drosophila) will produce a modification in genetically ivory wasps when injected into larvae. Why does an orange or ivory Habrobracon larva, feeding on an Ephestia host, not obtain sufficient cn^+ substance to modify its eye color; in other words, how, under these conditions, can there be an orange or ivory character in Habrobracon? Several explanations are possible; for example, it is possible that cn^+ substance is not present in the blood of paralyzed Ephestia larvae or that it is not effective when taken in with food material as it is in Drosophila. Another possibility, and one that we consider more probable, is as follows: It has been postulated that both v^+ and cn^+ substances are inactivated in air by enzymic oxidation.[9] If this is correct, the enzyme system concerned is present in Drosophila larvae and pupae.

It is probable, then, that this system is likewise present in Ephestia larvae. The parasitic Habrobracon larvae, according to this view, removes both cn^+ substance and the enzyme system from the host and it is at least possible that under these conditions the cn^+ substance obtained is inactivated in the intestine of the wasp larva.

Among more than twenty-five eye color characters studied in *Drosophila melanogaster*, differentiated from wild type by as many non-allelic genes, only one is both non-autonomous and differentiated from wild type by a marked deficiency (or absence) of cn^+ substance.[15] Among the known eye color characters in Habrobracon, only the orange series is similarly non-autonomous and characterized by a deficiency or absence of a diffusible substance that gives the same reaction in Drosophila as does cn^+ substance. We are therefore tempted to suggest that the cn^+ and o^+ genes are homologous and that their mutant alleles represent parallel mutations. Similar arguments, though having a less substantial experimental basis, would suggest that the a^+ (A) gene in Ephestia is homologous with either the v^+ or the cn^+ gene in Drosophila. A more nearly adequate knowledge than we now have of the chemical processes involved in eye color development in these insects should provide a more satisfactory basis for these and similar inferences.

NOTE: After the manuscript of the present paper was submitted for publication, a paper appeared (E. Becker and E. Plagge, *Naturwiss.*, **25,** 809 (1937)) in which it is shown that the red eyed mutant of Ephestia (a) is deficient in cn^+ substance (tested in Drosophila) and that wild type and cinnabar Drosophila contain a substance that modifies the red eyed mutant of Ephestia toward wild type. Although the direct test of the a mutant of Ephestia for v^+ substance remains to be made, it appears probable from the evidence now available that the a^+ (A) gene in Ephestia and the v^+ gene in Drosophila are homologous and that the recessive alleles (a and v) of these genes represent parallel mutations.

* That part of this work carried out at Stanford University was aided by funds granted by the Rockefeller Foundation.

[1] E. Caspari, *Arch. Entw.-mech.*, **130,** 353–381 (1933); A. Kühn, E. Caspari and E. Plagge, *Nachr. Ges. Wiss. Göttingen*, **2,** 1–29 (1935).

[2] H. Kikkawa, *Zool. Mag.* (Japan), **49,** 348–353 (1937).

[3] A. H. Sturtevant, *Proc. Sixth Int. Cong. Genet.*, **1,** 304–307 (1932); B. Ephrussi and G. W. Beadle, *Bull. Biol. Fr. Belg.*, **71,** 54–74 (1937); G. W. Beadle and B. Ephrussi, *Genetics*, **22,** 76–86 (1937).

[4] P. W. Whiting, *Biol. Bull.*, **63,** 296–309 (1932); P. W. Whiting and A. R. Whiting, *Jour. Genet.*, **29,** 311–316 (1934).

[5] B. Ephrussi and M. H. Harnly, *C. R. Acad. Sci., Paris*, **203,** 1028 (1936).

[6] E. Plagge, *Nachr. Ges. Wiss. Göttingen*, **2,** 251–256 (1936).

[7] Experiments as yet unpublished suggest that the so-called "ca^+ substance" of Drosophila[15] does not exist as a distinct substance but that what may be called the "claret effect" is one aspect of the action of either v^+ or cn^+ substance.

[8] B. Ephrussi, *Amer. Nat.* (in press).

[9] K. V. Thimann and G. W. Beadle, these PROCEEDINGS, **23,** 143–146 (1937).

[10] G. W. Beadle and L. W. Law, *Proc. Soc. Exptl. Biol. Med.* (in press).

[11] B. Ephrussi and G. W. Beadle, *Bull. Biol. Fr. Belg.*, **69**, 492–502 (1935). Also unpublished results.

[12] Y. Khouvine, B. Ephrussi, and M. H. Harnly, *C. R. Acad. Sci. Paris*, **203**, 1542 (1936).

[13] E. Becker, *Naturwiss.*, **25**, 507 (1937).

[14] B. Ephrussi and S. Chevais, these PROCEEDINGS, **23**, 428–434 (1937).

[15] G. W. Beadle and B. Ephrussi, *Genetics*, **21**, 225–247 (1936).

THE EFFECT OF pH ON THE DEVELOPMENT OF ULTRA-CENTRIFUGED FUCUS EGGS[1]

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It has been shown (Whitaker[2]) that the developmental polarity of fertilized eggs of *Fucus furcatus* is determined by centrifuging at 3000 x g. for 15 or 20 minutes. When the stratified eggs are reared in the dark in normal sea water (pH 7.9–8.0), more than 99% form rhizoids on the centrifugal halves of the eggs, and 93–99% do so within 10° of the centrifugal pole.

Beams[3] ultra-centrifuged fertilized eggs of *Fucus serratus* at 150,000 x g. for half an hour and found the polarity to be unaffected by stratification of the visible cell inclusions. It is possible but not probable that the difference lies in the two species of *Fucus*. It also appeared possible that the effects of centrifuging at 3000 x g. might be lost at 150,000 x g. Might some substance or structure be moved at 3000 x g. but be broken down at 150,000 x g.? Thousands of *F. furcatus* eggs were ultra-centrifuged in quartz centrifuge tubes at various forces including 150,000 x g. and 200,000 x g. for various durations, including 5 minutes and half an hour. The eggs were grown in normal sea water (pH 7.8–8.1) in the dark in a constant temperature room at 15°C. in thin cultures to avoid group effect (Whitaker[4]). Invariably 99% formed rhizoids on the centrifugal halves of the eggs, about 90% or more doing so within 10° of the centrifugal pole. The results are therefore essentially the same at 150,000 x g. and at 3000 x g., except that at the higher force 5 minutes of centrifuging is more than adequate to sharply stratify the eggs and determine polarity.

A number of environmental factors affect the determination of polarity in the *Fucus* egg.[2,4] When ultra-centrifuged eggs of *F. furcatus* are illuminated from one side during development, the polarity is affected by the direction of the light as well as by the stratification[5] so that in a population rhizoids are observed in all positions with respect to the stratification. The polarity of ultra-centrifuged eggs is also altered by the group effect, if

neighboring eggs are close together, especially if the sea water is somewhat acid. It will be shown below that the response of an isolated egg to its internal stratification depends on the pH of the medium. Beams does not indicate the conditions of his experiments with respect to these factors, and one or a combination of these factors would be adequate to explain why he observed rhizoids in all positions with respect to stratification.

The principal purpose of this communication is to present the results of experiments designed to test the effect of the pH of the external medium on the polarity of centrifuged *Fucus* eggs. A more detailed report will be published elsewhere. Eggs were fertilized and centrifuged in filtered normal sea water. They were centrifuged for 5 minutes at 150,000 x g., beginning 10–20 minutes after fertilization, and were then grown individually in 1 cc. sea water in small individual syracuse dishes in a dark constant temperature room at 15°C. Three hundred and ninety-six eggs were grown in filtered normal sea water, initially at pH 7.9–8.1, and 339 eggs were grown in sea water acidified to pH 5.8–6.1 with McIlvaines' buffer. One hundred per cent of the eggs reared at pH 7.9–8.1 formed rhizoids on the centrifugal halves. Only 10% of the eggs reared at pH 5.8–6.1 formed rhizoids on the centrifugal halves and 90% formed rhizoids instead on the centripetal halves. A considerable number formed rhizoids at the centripetal pole. The developmental response of the egg to its internal stratification is thus reversed with change in external pH. Centrifuged eggs reared at pH 6.0 in mass cultures which were thin so that mutual influences were reduced, but not absent, also statistically showed the reversal, but to a lesser extent.

Only tentative interpretations can be suggested at the present time. It was pointed out earlier[4,6] that the responses of the *Fucus* egg to a variety of agents could be interpreted on the basis of plant growth hormone (auxin) as one of the links in the reaction chain leading to rhizoid formation. The auxin interpretation is strengthened by the recent work of du Buy and Olson[7] who report having extracted growth substance from *Fucus* eggs. Olson and du Buy[8] also carried out experiments from which they conclude that the rhizoid forms on the side of the egg to which beta-indole acetic acid (hetero-auxin) or its potassium salt is applied in sufficiently high concentration. Auxins are active in the molecular form, and hydrogen ions convert dissociated auxin into molecular auxin. If auxin in the egg is concentrated at the centrifugal end as a result of being adsorbed or attached to larger particles which are moved by centrifuging, the behavior of centrifuged eggs at pH 8.0 can be understood. The reversed response at pH 6.0 could perhaps be explained on this basis by supposing that after acid activation the active auxin at the centrifugal pole is now present in such high concentration as to be inhibitory so that the rhizoid forms at a region of lesser auxin concentration, i.e., more centripetally. Another possibility is that

an amphoteric phenomenon is responsible for the reversal, either through its effect on auxin or through more direct effect on the underlying rhizoid forming processes. Auxin may be unmoved by the centrifuge and amphoteric substances (e.g., protein) concentrated at the centrifugal end may affect either the activity or transport of auxin in a manner which would reverse on either side of the isoelectric point of the amphoteric substances. Both internal pH and electrical gradients might be expected to reverse when the isoelectric point is crossed if amphoteric substances are concentrated at one end of the cell. Experiments are in progress which it is hoped may throw further light on these questions.

[1] This work has been supported in part by funds granted by the Rockefeller Foundation. The author is indebted to Dr. E. W. Lowrance for assistance in carrying out the experiments.

[2] Whitaker, D. M., *Biol. Bull.*, **73**, 249 (1937).

[3] Beams, H. W., *Jour. Marine Biol. Assn.*, **21**, 571 (1937).

[4] Whitaker, D. M., *Jour. Gen. Physiol.*, **20**, 491 (1937).

[5] As shown earlier in *Cystosira* with low centrifugal force by Knapp, E., *Planta*, **14**, 731 (1931).

[6] Whitaker, D. M., and E. W. Lowrance, *Jour. Gen. Physiol.*, **21**, 57 (1937).

[7] du Buy, H. G., and R. A. Olson, *Am. Jour. Bot.*, **24**, 609 (1937).

[8] Olson, R. A., and H. G. du Buy, *Ibid.*, **24**, 611 (1937).

CHROMOSOME NUMBERS IN NODULES AND ROOTS OF RED CLOVER, COMMON VETCH AND GARDEN PEA[1]

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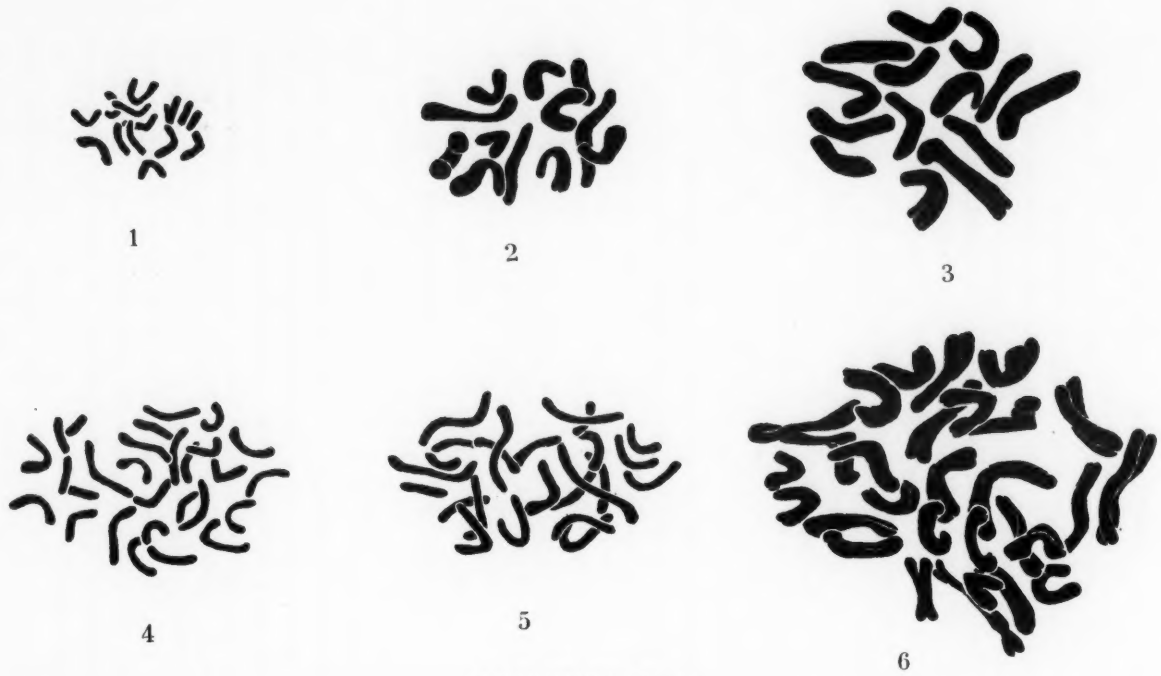
An observation of 28 chromosomes ($4n$) on somatic equatorial plates in the meristematic region of the nodule of red clover instead of the 14 chromosomes ($2n$) found in root tip cells suggested the present investigation. The number of chromosomes characteristic of nodular tissue has heretofore been reported in two instances. Milovidov[2] found 42 chromosomes in cells of the cortex of the nodule of *Lupinus mutabilis*. Lechtova-Trnka[3] observed approximately 16 chromosomes in nodular material of both *Sophora Moorcroftiana* and *Robinia viscosa*. The exact number was difficult to determine because of the large size and crowded condition of the chromosomes on the equatorial plate.

Nodules and root tips of red clover (*Trifolium pratense* L.), common vetch (*Vicia angustifolia* L.) and garden pea (*Pisum sativum* L.) were used in the present study. They were obtained from field-grown plants and from



EXPLANATION OF PLATE

Figures 1–3. Somatic chromosomes from root tip cells. Fig. 1—Red clover ($2n = 14$). Fig. 2—Common vetch ($2n = 12$). Fig. 3—Garden pea ($2n = 14$).

Figures 4–6. Chromosome complements from nodule tissue. Fig. 4—Red clover (28 chromosomes). Fig. 5—Common vetch (24 chromosomes). Fig. 6—Garden pea (28 chromosomes). × 2150.

plants grown in the greenhouses at the University of Wisconsin. Of the latter plants, some were grown in sterilized soil with the addition of controlled nutrients. The seeds were sterilized before planting and the young plants inoculated with an effective strain of the proper *Rhizobium* at the time of germination. The remaining plants were grown in an ordinary greenhouse soil mixture and, as with the field-grown material, the nodules resulted from chance inoculation.

Root tips and nodules of varying ages were fixed in Karpechenko's modification of Navashin's fluid and in Carnoy's alcohol-acetic-chloroform solution. The material fixed in Karpechenko's fluid was embedded in paraffin and sectioned. Aceto-carmin smears were made from that fixed in Carnoy's solution. The latter method was found to be more convenient for the determination of the chromosome numbers in the nodules. The mitotic spindles occur in irregular positions in the meristematic region, and the smear method makes it possible to obtain a higher percentage of polar views of equatorial plates than the sectioned material reveals. Transverse sections of root tips gave an abundance of figures wherein an exact count of the chromosomes was possible. Nodules from five plants of each species were examined (table 1).

TABLE 1

SHOWING NUMBER OF CHROMOSOMES IN THE NODULES OF RED CLOVER, COMMON VETCH AND GARDEN PEA

		RED CLOVER — $2n = 14$		COMMON VETCH — $2n = 12$		GARDEN PEA — $2n = 14$	
PLANT	NODULE	NUMBER OF COUNTS	NUMBER OF CHROMOSOMES	NUMBER OF COUNTS	NUMBER OF CHROMOSOMES	NUMBER OF COUNTS	NUMBER OF CHROMOSOMES
1	a	1	28	1	24	2	28
	b	9	28	4	24	2	28
	c	1	28	1	24	2	28
2	a	5	28	4	24	2	28
3	a	2	28	2	24	4	28
4	a	3	28	2	24	31	28
	b[1]	5	28	1	24		
						42[3]	14
5	a	1	28	7	24	2	28
	b	1	28	14	24	6	28
				1	12[2]		
Totals	9	28	28	36	24	51	28

[1] Of the garden pea only 8 nodules were used.

[2] One count showed 12 chromosomes.

[3] 73 chromosome counts were made from one nodule; 42 showed 14 chromosomes, 31 showed 28 chromosomes. See text for explanation.

The somatic number of chromosomes of red clover as observed in the dividing cells of the root tip is 14 (figure 1), whereas that of the cells in the nodule is 28 (figure 4). Counts were made in aceto-carmin preparations from 9 nodules (table 1). Polar views of 28 equatorial plates in nodular

tissue revealed regularly the tetraploid condition. Anaphase figures showed 28 chromosomes passing to each pole. In no case as yet has other than the tetraploid number appeared.

The diploid chromosome number in the vetch is 12 (figure 2). Twenty-four chromosomes are usually present on equatorial plates in the dividing cells of the nodule (figure 5). A total of 36 counts taken from 9 nodules revealed this number (table 1). Only one figure was observed with the diploid number (12).

Fourteen chromosomes are present in root tip cells of the pea (figure 3). The tetraploid number (28) was observed on 51 equatorial plates in 8 nodules (figure 6). One nodule having a large number of dividing cells showed both diploid and tetraploid complements (table 1). The tetraploid figures were in cells whose cytoplasm stained deeply whereas the cytoplasm surrounding the diploid equatorial plates took up little of the stain. Transverse sections of the apical meristematic portion of a pea nodule reveals a definite arrangement of the two types of cells. The central portion of the nodule is composed of tetraploid cells. This is surrounded by several layers of cortical cells which are diploid. Median sections of the nodule show a central cylinder of large cells packed with bacteria. There is no evidence of the presence of bacteria in the smaller cells of the cortical region. The nuclei of the invaded cells, also, are distinctly larger than those of the cortex. Nuclear and cell size are commonly, as shown by Gates[4] and others, increased with an increase in the chromosome number.

The cells of stimulated growths and hypertrophies of various types have, in some instances, been found to possess chromosome numbers differing from that typical of the species. Tetraploid tomato and Solanum plants have been produced from budding callus tissue, a fact taken to indicate that the cells of such tissue may oftentimes be tetraploid rather than diploid (Jørgensen;[5] Lindstrom and Koos[6]). Diploid, tetraploid and octoploid cells have been observed in crown gall tissue of the sugar beet (Winge[7]) and of beet and tobacco (Levine[8]). Kostoff and Kendall[9] find polyploid cells in crown gall tissue, in spontaneous tumors on hybrid tobacco plants and in tumors caused by chemical substances. Whitaker[10] reports an occasional tetraploid condition in nonparasitic tumors on grafted tobacco. A varying number of chromosomes ranging from haploid to tetraploid or even higher is present in the cells of carcinomatous tissue in the fowl, rat and man (Levine[8]). In comparison with these findings, observations thus far indicate that in nodules of red clover, common vetch and garden pea the infected cells are regularly tetraploid.

[1] Herman Frasch Foundation in Agricultural Chemistry, Frasch Paper No. 148. Joint contribution from the Departments of Agricultural Chemistry and Agricultural Bacteriology and from the Department of Genetics, Agricultural Experiment Station,

No. 227, and the Department of Botany, University of Wisconsin. Published with the approval of the Director of the Station.

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GROUPS HAVING A MAXIMUM SET OF INDEPENDENT GENERATORS OF THE SAME ORDER

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Suppose that a given group G has a maximum number set of independent generators, that is, a set which involves as many operators as there are prime factors in the order of G. Obviously each of the operators of such a set is of prime order.[1] It is known that these operators can always be so selected that their product is equal to the order of G, and when they have been so selected and the order of G is not a power of a prime number then they can be separated into distinct subsets such that each subset generates a Sylow subgroup of G and that each of these Sylow subgroups is abelian and of type 1^k. When G is non-abelian only the Sylow subgroup whose order is a power of the largest prime number which divides the order of G is always invariant under G, and when all the operators in a maximum number set of independent generators of G are of the same order this order is the smallest prime number which divides the order of G.

Suppose that G contains a maximum number set of independent generators of the same order. We proceed to prove that all the operators of larger prime orders contained in G are relatively commutative and hence they generate an invariant abelian subgroup of G. To prove this fact let $s_1, s_2, \ldots, s_l$ be a subset of the given maximum number set of independent generators of G such that this subset generates a Sylow subgroup of G. Every other operator in the given maximum number set when adjoined to this subset gives a set of generators of a subgroup of G whose order is divisible by a larger prime number p than the prime divisors of the order of the given Sylow subgroup. Hence this subgroup contains an operator t_1 of order p. Similarly we find another operator t_2 of prime order $q \geqq p$. To

prove that t_1 and t_2 are commutative we may assume that $s_1 s_\gamma = t_1$ and that $s_2 s_\delta = t_2$ and consider the group generated by the four operators $s_1, s_2, s_\gamma, s_\delta$ contained in the given maximum number set of independent generators of G.

The order of this group is the product of four prime numbers and the group involves the two operators t_1 and t_2. Two of these prime numbers are p and q and two of them divide the order of the given Sylow subgroup. Hence t_2 is transformed into a power of itself by each of the three operators s_1, s_2, s_γ. If this is not the first power its index belongs to an exponent which is equal to the common order of these operators. The product $s_1 s_\gamma$ cannot transform t_2 into a power whose index belongs to the same prime number since the order of $s_1 s_\gamma$ is a larger prime number. Hence it results that t_1 and t_2 are commutative. That is, *when all the operators of a maximum number set of independent generators of a group have the same order then all the operators of the group whose prime orders exceed this common order are relatively commutative and generate an invariant abelian subgroup of the group.*

From the preceding paragraphs it results that comparatively few of the groups which have a maximum number set of independent generators can also have such a set in which all the operators have the same order. Every abelian group whose independent generators are composed of operators of odd prime orders can be extended in at least one way so as to obtain a group which has a maximum number set of independent generators of the same order. One such extending operator is of order 2 and transforms every operator of this abelian group into its inverse. When there exists a prime number such that each prime factor of the order of this abelian group is congruent to unity with respect to this prime number then the extending operator may clearly have an order which is equal to this prime number. Such extensions can obviously be effected in various ways whenever the order of this abelian subgroup is divisible by more than one prime number.

Suppose now that G has a maximum number set of independent generators composed of operators of the same order p and that it contains a Sylow subgroup of this order which is generated by s. A maximum number set of independent generators of G which is such that the product of the orders of these generators is equal to the order of G contains then only one operator of order p while every one of its other operators is of higher order. Each of these other operators is the product of s and some operator of order p contained in the given maximum number set of independent generators. Since each of the operators of a new maximum number set of independent generators found in this way is transformed into powers of itself by s and all these operators besides s are relatively commutative there results the following theorem: *If a group has a maximum number set of independent generators composed of operators of the same order* p *and contains a Sylow subgroup of order* p *then it involves an invariant abelian subgroup whose inde-*

pendent generators are of prime orders such that each order is congruent to unity modulo p *and all of the remaining operators are conjugate under the group.*

While the smallest Sylow subgroup of a group which has a maximum number set of independent generators whose common order is p is obviously of order p there is no largest such Sylow subgroup when the subgroup generated by its operators of larger prime orders is given. This results directly from the fact that if G is a given group which has a maximum number set of independent generators of order p then the direct product of G and any abelian group of type 1^k whose order is a power of p is also such a group. Such direct products will be excluded in what follows. When this is done and the invariant abelian subgroup which is generated by the operators of prime orders larger than p in G is fixed it can be proved that the order of the Sylow subgroup of G whose order is a power of p cannot exceed p^m, where m is the number of the independent generators of prime orders of the given abelian invariant subgroup of G.

To prove this theorem it may be noted that if $s_1, s_2, \ldots, s_l$ $(l > m)$ is a set of independent generators of a Sylow subgroup whose order is a power of p contained in G an additional independent generator of G is the product of one of these l operators and some other operator of order p contained in the given maximum number set of independent generators of G. These two operators generate a non-abelian subgroup of G whose order is the product of two prime numbers. We form such subgroups successively by using each time a different independent generator of the given Sylow subgroup of G, and continue this process until a set of m independent generators of prime orders greater than p of the given abelian invariant subgroup of G is found. The remaining $l - m$ independent generators of the Sylow subgroup of order p^l contained in G may be supposed to generate an invariant subgroup of G which has only the identity in common with the invariant subgroup generated by the set of $2m$ independent generators noted above.

The fact that the subgroup generated by these $l - m$ operators of the given maximum number set of independent generators of G is invariant under G can be proved by observing that it is the cross-cut of all the Sylow subgroups of G whose orders are powers of p. This may be proved by observing that when the given Sylow subgroup of order p^l contained in G is extended by an operator of order p not contained in this Sylow subgroup there results a group whose order is a prime number q times the order of this Sylow subgroup under which $l-1$ of the independent generators of the given Sylow subgroup may be assumed to be invariant. This group contains q Sylow subgroups of order p^l which have a cross-cut of index q under all of these Sylow subgroups. When G contains more than $l + 1$ independent generators this process can be repeated until we arrive at the given invariant cross-cut of order p^{l-m}.

Since the two given invariant subgroups have only the identity in common and the product of their order is equal to the order of G it results that G is the direct product of these two subgroups. This proves the following theorem: *If a group has a maximum number set of independent generators composed of operators of the same order* p *and if its invariant abelian subgroup which is generated by its operators of prime orders larger than* p *has* m *such generators then the order of its Sylow subgroup whose order is a power of* p *cannot exceed* p^m *unless the group is a direct product such that one of its factor groups is abelian and involves only operators of order* p *besides the identity.* It may be noted that when the order of this Sylow subgroup is p^m then the group is the direct product of m non-abelian groups such that each of these groups is of order pq, q being a larger prime number than p. Moreover, every such direct product has a maximum number set of independent generators composed of operators of order p.

If the operators of a maximum number set of independent generators of a group G are so chosen that the product of their orders is equal to the order of G then it is known that the operators of the same order in this set generate a Sylow subgroup of G for each of the orders. These operators of the same order can usually be selected in various ways. In the case when they generate the Sylow subgroup whose order is a power of the smallest prime number which divides the order of G they may be replaced by any other possible set of independent generators of this Sylow subgroup but this is not generally the case as regards the subset of independent generators which generate another Sylow subgroup of G since in this case it is not necessary that the group generated by an operator of such a subset is transformed into itself by all the operators of a smaller order in the given maximum number set of independent generators of G.

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GROUPS HAVING A MAXIMUM NUMBER SET OF CONJUGATE INDEPENDENT GENERATORS

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If a group G has a maximum number set of independent generators composed of operators which are conjugate under G then all the operators of the set are of the same order and hence such a group is included among those which have a maximum number set of independent generators composed of operators of the same order.[1] In particular, all the operators of the set have for their order the smallest prime factor p of the order G, and G

contains an abelian invariant subgroup whose independent generators are composed of operators of prime orders greater than p such that each of these prime numbers is congruent to unity modulo p. If this subgroup is so selected that it includes all of the operators of G of prime orders greater than p then all of the remaining operators of G are of order p and the cyclic subgroups of order p generated by them constitute a complete set of conjugates under G. In particular, the order of G is not divisible by p^2. Simple illustrative examples of such groups are the dihedral groups whose orders are the double of odd prime numbers.

From the preceding paragraph it results that if a group has a maximum number set of independent generators composed of operators which are conjugate under it then its Sylow subgroups which involve these operators are of prime order p. Moreover, if a group which has a maximum number set of independent generators composed of operators of the same order p has a Sylow subgroup of order p then it contains a maximum number set of independent generators composed of conjugate operators. On the other hand, if such a group contains a Sylow subgroup whose order is divisible by p^2 then it cannot contain a maximum number set of independent generators composed of conjugate operators since the order of its Sylow subgroups is the index under G of the subgroup generated by all its operators of prime orders greater than p. Hence there results the following theorem: *A necessary and sufficient condition that a group which has a maximum number set of independent generators of the same order has such a set in which all these operators are conjugate under the group is that it has a Sylow subgroup of this order.*

Two necessary and sufficient conditions that there is a group of a given composite order which has a maximum number set of conjugate independent generators are that this order is not divisible by the square of its smallest prime factor and that each of its other prime factors is congruent to unity with respect to this smallest prime factor. In particular, there is one and only one such group whose order is the double of an arbitrary odd number. This is the dihedral group whenever the order is not divisible by the square of an odd prime number and the generalized dihedral group whenever the order is divisible by such a square. When the order of G is odd and is not the product of two prime numbers there exists always more than one such group of this order but all of these possible groups are conformal. In one and only one of them each of the cycle subgroups of prime order greater than p is invariant under G.

When all the prime numbers which divide the order of G and exceed p are distinct and their number is n the number of these groups of a given order is equal to $(p-1)^{n-1}$. This results from the fact that an operator of order p to be adjoined to the given abelian subgroup which is generated by all the operators of G which are of prime order greater than p may be

assumed to transform one of these operators into an arbitrary power whose index belongs to an exponent which is equal to p. When some of the prime numbers greater than p which divide the order of G are equal to each other it is somewhat more difficult to determine the total number of these possible groups of a given order. In the special case when there are only two such equal prime factors of the order of G and this order is the product of three prime numbers it is not difficult to determine the number of these possible groups as follows: Let q be one of the two prime numbers which divide the order of G and exceed p while s is an operator of order p which is adjoined to the given invariant subgroup of order q^2.

It may be assumed that s transforms one of the two independent generators of order q into an arbitrary power of itself such that the index of this power belongs to exponent p modulo q. The other independent generator of order q of the given invariant subgroup of order q^2 is transformed by s into one of the possible $p - 1$ powers of itself. The indexes of the $p - 1$ distinct powers of this independent generator of order q which belong to exponent p modulo q together with the identity constitute a group of order p modulo q. Hence the $p - 1$ powers into one of which s may transform the second of the two given independent generators of order q after the first one has been transformed have indexes which constitute a cyclic group of order $p - 1$. As this has one and only one operator of order 2 the given second independent generator can be transformed in $(p + 1)/2$ ways relatively to the first. That is, *there are exactly* $(p + 1)/2$ *groups of order* pq^2, p *and* q *being prime numbers such that* $q - 1$ *is divisible by* p, *which separately have a maximum number set of conjugate independent generators.*

When the order of G is divisible by q^m, $m > 2$, but exceeds pq^n, the adjoined operator s transforms at least m of the cyclic subgroups of G separately into themselves and the subgroups of order pq^m are completely determined by the different powers into which s transforms the given m independent generators of order q. Such a group is obviously transformed into itself by operators which transform these m independent generators according to the symmetric group of degree m. A necessary and sufficient condition that s transforms each of the cyclic subgroups of order q in a subgroup of order q^k contained in G into itself is that s transforms each of the k operators in a set of independent generators of this subgroup into the same power of itself. If s transforms each of these k independent generators into the same power of itself but another independent generator of order q contained in G into a different power of itself then the group generated by these $k + 1$ independent generators of G contains one and only one invariant subgroup of order q besides those found in a given subgroup of order q^k.

It may be noted that while all of these groups of the same order are conformal there are also usually many of them which are not only con-

formal but have also the same number of conjugate cyclic subgroups of the same order. This is the case, for instance, as regards all except one of the $(p-1)^{n-1}$ groups of the same order noted above. We have therefore here systems of distinct groups which have a large number of properties in common besides being conformal. The number and the form of the operators of possible sets of independent generators are thus seen to throw considerable light on certain systems of groups of finite orders.

[1] Miller, G. A., *Proc. Nat. Acad. Sci.*, **23**, 333–337 (1937).

THE EXISTENCE OF A MINIMAL SURFACE OF LEAST AREA BOUNDED BY PRESCRIBED JORDAN ARCS AND PRESCRIBED SURFACES

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Plateau's problem with non-fixed boundaries can be solved by means of a general theorem concerning boundary values of harmonic functions. The following note proves this theorem and, as a consequence, the existence of a minimal surface of least area of the type of a circular disc bounded by a given Jordan arc and a closed portion of a continuous surface. The generalization to cases where the boundary may consist of any finite number of Jordan arcs and of point sets on different manifolds, and where the topological structure of the minimal surface is arbitrarily prescribed, will be given in a more detailed paper.[1]

1. *Lemma.*—We consider a system of harmonic functions $x_i(u, v)$, $i = 1, \ldots, n$, regular in the upper half plane $B: v > 0$, so that the "Dirichlet integral" for the potential vector $\mathfrak{x}$ with the components x_i

$$D[\mathfrak{x}] = \tfrac{1}{2}\int\int_B (\mathfrak{x}_u^2 + \mathfrak{x}_v^2)dudv$$

is finite. Then there exists a quantity $t(h)$ and a quantity $\sigma(h)$ with

$$t(h) \to \infty \text{ and } \sigma(h) \to 0 \text{ for } h \to 0 \tag{1}$$

so that for each point u_0, v_0 in B at the distance $v_0 = h$ from the u-axis

$$|\, \mathfrak{x}(u, h) - \mathfrak{x}(u_0, h)\,| \leq \sigma(h) \tag{2}$$

holds for the whole segment S on $v = h$ defined by

$$| u - u_0 | \leq h\, t(h). \tag{3}$$

Proof: If

$$D_h[\mathfrak{x}] = \epsilon(h)^4.$$

denotes the Dirichlet integral of $\mathfrak{x}$ over the strip $0 < v < 2h$, we have $\epsilon(h) \to 0$ for $h \to 0$ because of the existence of $D[\mathfrak{x}]$. Let K_h be the circle of radius $\frac{1}{2}h$ around a point $u, v = h$ on the segment S; then we have by the mean value theorem of potential theory

$$\mathfrak{x}_u(u, h) = \frac{4}{h^2\pi} \int\int_{K_h} \mathfrak{x}_u du dv$$

and hence by Schwarz's inequality

$$\mathfrak{x}_u(u, h)^2 \leq \frac{16}{h^4\pi^2} \cdot \frac{h^2\pi}{4} \int\int_{K_h} \mathfrak{x}_u{}^2 du dv \leq \frac{8}{h^2\pi}\, \epsilon^4(h) < \frac{9}{h^2}\, \epsilon^4(h).$$

This gives

$$|\mathfrak{x}(u, h) - \mathfrak{x}(u_0, h)| \leq \int_{u_0}^{u} |\mathfrak{x}_u(u, h)|\, du \leq |u - u_0|\, \frac{3}{h}\, \epsilon^2(h)$$

and therefore the Lemma follows with

$$t(h) = \frac{1}{\epsilon(h)}, \quad \sigma(h) = 3\epsilon(h).$$

2. *Theorem.*—Let $\mathfrak{x}_\nu(u, v)$ be a sequence of potential vectors regular for $v > 0$ with equally bounded Dirichlet integrals

$$D[\mathfrak{x}_\nu] \leq \tfrac{1}{2}L.$$

We suppose the boundary values of $\mathfrak{x}_\nu$ are on a closed manifold M_ν which means the distance of the point $\mathfrak{x}_\nu(u, h)$ to M_ν tends to zero with h—necessarily uniformly in u. We further suppose that for $\nu \to \infty$ the longest distance of points in M_ν to a closed manifold M tends to zero. We finally assume that $\mathfrak{x}_\nu$ converges to a potential vector $\mathfrak{x}$ uniformly in each closed subdomain of the half plane B.[3] Then the boundary values of $\mathfrak{x}$ are on M.

Proof: With the denotation of the Lemma we have on the straight segment $S: v = h$ and $|u - u_0| \leq h\, t(h)$ the inequality (2). Because of the uniform convergence of $\mathfrak{x}_\nu$ to $\mathfrak{x}$ on S we have for sufficiently large $\nu > \nu(h)$ on S:

$$|\mathfrak{x}(u, h) - \mathfrak{x}_\nu(u, h)| \leq \sigma(h),$$

hence on S:

$$|\mathfrak{x}(u_0, h) - \mathfrak{x}_\nu(u, h)| \leq 2\sigma(h)$$

and therefore for every δ with $0 < \delta < h$

$$|\mathfrak{x}(u_0, h) - \mathfrak{x}_\nu(u, \delta)| - 2\sigma(h) \leq |\mathfrak{x}_\nu(u, h) - \mathfrak{x}_\nu(u, \delta)|$$

and thus

$$|\mathfrak{x}(u_0, h) - \mathfrak{x}_\nu(u, \delta)| \leq 2\sigma(h) + \int_\delta^h \left| \frac{\partial \mathfrak{x}_\nu}{\partial v} \right| dv. \tag{4}$$

If $d_\nu(u, h)$ denotes the distance of the point $\mathfrak{x}_\nu(u, h)$ to M_ν, and $d(u, h)$ the corresponding distance to M, we infer from (4) for sufficiently small δ, so that $d_\nu(\mu, \delta) < \sigma(h)$,

$$d_\nu(u_0, h) \leq 3\sigma(h) + \int_\delta^h \left| \frac{\partial \mathfrak{x}_\nu}{\partial v} \right| dv.$$

Integrating over S with respect to u and using Schwarz's inequality

$$\left(\int\int_R \left| \frac{\partial \mathfrak{x}_\nu}{\partial v} \right| du dv \right)^2 \leq h^2 t(h) \int\int \left(\frac{\partial \mathfrak{x}_\nu}{\partial v} \right)^2 du dv \leq h^2 t(h) L$$

for the rectangle

$$R : |u - u_0| \leq h\, t\,(h) \text{ and } 0 < v \leq h,$$

we have

$$ht(h) d_\nu(u_0, h) \leq 3h\, t(h)\sigma(h) + (h^2\, t(h) L)^{\frac{1}{2}}$$

or

$$d_\nu(u_0, h) \leq 3\sigma(h) + \frac{1}{\sqrt{t(h)}} \sqrt{L}.$$

For $\nu \to \infty$ it follows immediately

$$d(u_0, h) \leq 3\sigma(h) + \frac{1}{\sqrt{t(h)}} \sqrt{L}$$

which proves our theorem on account of (1).

The following supplementary facts, not needed here but useful for other applications, may be noted:

COROLLARY 1. A corresponding theorem holds for domains B bounded by k circles.

COROLLARY 2. If $\mathfrak{x}$ is not constant, in the passage to the limit additional boundary conditions in the form of inequalities are preserved, e.g., if a part of the u-axis by virtue of $\mathfrak{x}_\nu$ corresponds monotonically to a Jordan arc γ_ν tending to a Jordan arc γ, then by $\mathfrak{x}$ the same part of the u-axis corresponds monotonically to γ.

COROLLARY 3. If M_ν and M extend to infinity but are closed in every closed spherical domain in the $\mathfrak{x}$-space, the theorem subsists except possibly for values u_0 which form a set of measure zero.

COROLLARY 4. The theorem subsists, if instead of the relations defining M_ν and M, we have relations between the coördinates x_i which in addition contain explicitly the coördinate u on the u-axis.

3. *The Plateau Problem.*[4]—In the n-dimensional $\mathfrak{x}$-space a closed manifold M may consist of a part of a surface T of $n-1$ or less dimensions and of a Jordan arc γ joining two points A and B on T. We consider all surfaces $\mathfrak{x}(u, v)$ defined in the upper half plane $B: v > 0$, so that $\mathfrak{x}$ is continuous and has piecewise continuous first derivatives in B and has the boundary values on M whereby to the segment $|u| \geq 1$ of $v = 0$ the arc γ may correspond in a continuous and monotonic way. We suppose that there exist admissible vectors with finite Dirichlet integral $D[\mathfrak{x}]$. Then the problem is to prove the existence of an admissible $\mathfrak{x}$ for which the absolute minimum d is attained. From the paper quoted above it then follows directly that the solution represents a minimal surface.

We consider a minimizing sequence of potential vectors $\mathfrak{x}_\nu$ for which the boundary values of $\mathfrak{x}_\nu$ are on manifolds M_ν consisting of γ and a continuous arc γ_ν', which tends to T with increasing ν. Because of the boundedness of $D[\mathfrak{x}_\nu]$ we can choose a subsequence $\mathfrak{x}_\nu$ converging, as in the theorem of Nr. 2, to a potential vector $\mathfrak{x}$ with $D[\mathfrak{x}] \leq d$. By imposing a three point condition, or a similar condition, on $\mathfrak{x}_\nu$, we can prevent $\mathfrak{x}$ from being constant. As is seen, e.g., from the consideration of the previous papers, or from Corollary 2, $\mathfrak{x}$ maps the segment $|u| \geq 1$ of the u-axis monotonically on γ, and the theorem of Nr. 2 shows that all the other boundary points of $\mathfrak{x}$ are on T. Hence $\mathfrak{x}$ is admissible, we have $D[\mathfrak{x}] = d$, and $\mathfrak{x}$ solves our problem.

4. *Remarks.*—(*a*) If the surface T extends to infinity it may happen that the solution of the problem and even all the vectors of a minimizing sequence are not bounded but have a finite or even infinite number of spikes reaching to infinity. Our theory can be extended to such cases by means of Corollary 3 of Nr. 2.

(*b*). If the whole manifold M reduces to a Jordan curve, we come back again to the ordinary Plateau problem. Our theorem thus leads to a new version of the solution of this problem. However, it is an interesting fact that, in this case, one has to require expressly the monotonic character of the mapping of the u-axis to the simply described Jordan contour.

(*c*) The generality of the manifolds M considered in this note excludes a detailed analysis of the behavior of the minimal surfaces in the boundary points on T. The variational equation leads to a "natural boundary condition" expressing orthogonality in an average sense. But to prove, under suitable additional conditions for M, the existence and the strict orthogonality of a tangent plane in boundary points of S on M seems a problem not accessible to the methods of this note.[5]

[1] This problem corresponds to Douglas' generalization of Plateau's original problem, cf. the papers by Douglas, *Journ. Math. Phys.*, **15**, 55 ff (Feb., 1936) and 106 ff (June, 1936) and Courant, these PROCEEDINGS, June, 1936, 369 ff and *Ann. Math.*, **38**, 679 ff (1937). For the case of boundary planes, cf. also the doctoral thesis by E. Ritter, soon to appear.

[2] It is noteworthy that the Lemma and the following Theorem subsist if instead of harmonic functions general monotonic functions of u and v are admitted.

[3] Incidentally, at least a subsequence of the ξ_ν satisfying this last condition can always be found.

[4] A knowledge of the first two sections of the paper in the *Ann. Math.* (see footnote 1) is required for the following.

[5] Cf. the similar situation with the boundary value problem of elliptic partial linear differential equations. See Courant-Hilbert, *Meth. math. Physik*, Vol. II, Chapter 7.

THE CONTINUUMPROBLEM

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1. The continuumhypothesis of Georg Cantor consists in the theoretical assumption that the continuum represents the second infinite potency following the potency of the enumerable set of the natural numbers in immediate succession.

In terms of the concept of Alephs, discovered by Cantor in an attempt to build up a system of infinite potencies in which immediate succession is secured, the hypothesis can be stated in the form of the equation

$$2^{\aleph_0} = \aleph_1.$$

It is well known that this problem has played the rôle of the central problem in the theory of aggregates as developed by Georg Cantor. D. Hilbert in 1900 has restated the problem and he gave later an exposition of his ideas concerning the problem in a paper constituting a different approach which has not yet led to a conclusive proof.

The object of the present paper is to offer a solution of the problem within a new system of postulates of the theory of aggregates. It will be shown that by adoption of two new postulates which we call the axiom of identity and the postulate of exclusion a proof of the continuumhypothesis can be given.

We accept as basis of the deductions, beside these postulates any of the established systems of the theory of aggregates (Zermelo, Adolf Fraenkel, v. Neumann and others).

2. We formulate the new postulates in the following way:

I. *The Axiom of Identity.*—Be M_1 a subset of the set M. If in our system no proof can exist that M_1 is a true subset of M, we assume identity of M_1 and M.

II. *Postulate of Exclusion.*—Be M_1 a subset of M. If the following conditions are fulfilled

a) M_1 is equivalent to M,

b) M_1 is of the same ordertype as M,

c) no element of the subset M_2 complementary to M_1 in M is given,

then we assume that no proof is possible by some fourth argument, that M_1 is a true subset of M.

If therefore the conditions *a*) *b*) *c*) are fulfilled we conclude with the aid of the axiom of identity that M_1 is identical with M.

We do not comment in this paper on the question of independence of these axioms from the system of Zermelo-Fraenkel, neither do we discuss here the compatibility of the new axioms with the old ones. We confine ourselves to the more immediate task to prove that these axioms will be sufficient to solve the continuumproblem, and reduce it to its axiomatic equivalent.

It shall, however, be noticed that the postulate of exclusion is sufficient to prove that the continuumhypothesis is true or independent. The axiom of identity excludes the latter possibility.

3. Be

$$\alpha_1, \alpha_2, \ldots, \alpha_s, \ldots \tag{1}$$

an enumerable sequence of numbers of the second class. The set T of all sequences (1) is equivalent to the continuum.

We introduce now a number of concepts and theorems which explain under which conditions the existence of sequences (1) different from the sequences of a given system t can or cannot be derived.

3.1. *Distinctionsets.* Be t a subset of the set T. Be (τ_s) a sequence different from each sequence of t. In each sequence of t there shall exist therefore at least one element ϵ_s different from the element with the same index s of the sequence (τ_s).

We call the element ϵ_s a distinctionelement.

A set D of distinctionelements ϵ_s which contains at least one distinctionelement in each sequence of t, is called a sufficient distinctionset with regard to t.

D fulfills the following conditions:

(*a*) D contains at least one element in each sequence.

(*b*) the elements of D with the same index s form a subset D_s which does not have as element every number of the second class.

It follows immediately the THEOREM 1:

The existence of a distinctionset D fulfilling (a) and (b) is the internal condition with regard to the set t which has to be fulfilled, if a sequence (τ_s) different from every sequence of t shall exist. The condition is necessary and obviously sufficient.

3.2. *Overalldense Sets.*—We call the subset t of T overall dense in T if for every given number α of the second class a sequence of t exists with every element greater than α.

THEOREM 2. Every sufficient distinctionset D of an overall dense subset t contains numbers of the second class greater than any given number α of the class.

Since D contains elements of each sequence, the sequence in which each element is greater than α has an element in common with D which greater than α.

THEOREM 3. Under the same assumptions at least one index r exists so that D_r contains elements greater than any given α.

Proof: If indeed in the contrary no such r would exist, and α_s would be the upper limes of the numbers in D_s, the upper limes α of all α_s would be greater than any element of D contrary to Theorem 2.

THEOREM 4. Be M_{1r} the set of numbers contained in D_r and be M the set of all numbers of the second class. We have

$$a)\ M_{1r} \text{ equivalent } M$$

and

$$b)\ M_{1r} \text{ of same ordertype as } M.$$

Proof: a) Since D_r contains elements greater than any given α, it follows that M_{1r} is of power $\aleph_1$. b) M_{1r} is a well ordered subset of the well ordered set M which is of minimum ordertype of his powerclass. Hence M_{1r} is also of minimum ordertype, and therefore of the ordertype of M.

3.3 Suppose that an overalldense subset t of T exists, for which no knowledge is given that a sequence (τ_s) exists which is different from all sequences of t. We state the

THEOREM 5. Under the assumptions made we have for at least one index

$$M_{1r} \equiv M$$

and

$$t \equiv T.$$

Proof: We assume that in the contrary a proof is possible that t is a true subset of T. Then a system of distinctionsets D should be defined fulfilling the conditions 3.1a) and b).

Because of 3.2 Theorem 4, for at least one index r, D_r exists so that the set M_{1r} fulfills the conditions a) and b) of the axiom of exclusion.

On the other hand no sequence (τ_s) different from all sequences of t is given. This means that no τ_s is given for any value of the index s, and in particular not for $s = r$.

Indeed the knowledge of some of the τ_s is meaningless, if it does not imply that they form a part of a sequence which is different from all sequences of t: the knowledge of such a sequence forms an undivisible unit. Hence we have in addition to *a*) and *b*):

c) a number τ_r different from all numbers of D_r, respectively. M_{1r}, is not known. M_{1r} fulfills, therefore, the conditions of the axiom of exclusion and therefore is $M_{1r} \equiv M$. In consequence no D_r can be found fulfilling 3.1*b*) so that D cannot be determined. No proof, therefore, is possible that the set t is a true subset of T and because of the axiom of identity we assume $t \equiv T$.

4. THEOREM 6. The power of the set of the overalldense sets t which are of power $\aleph_1$ is $2^{\aleph_1}$.

Since the power of all subsets t of T of power $\aleph_1$ is $(2^{\aleph_0})^{\aleph_1} = 2^{\aleph_1}$ it is sufficient to prove that the set P of overalldense sets in which not more than one sequence occurs with the lower limit α, is already of power $2^{\aleph_1}$.

Be $p\alpha$ the set of all sequences of lower limit α. The product P of the $\aleph_1$ sets $p\alpha$ is certainly not smaller than $2^{\aleph_1}$ and therefore of power $2^{\aleph_1}$.

Existence of overalldense sets of power $\aleph_1$ for which no knowledge is given that they are true subsets of T:

Applying the axiom of choice on the product $P^{\aleph_1}$ times, and adding the $\aleph$ choice sets, we can select an overalldense set t of power $\aleph_1$ which is sufficiently arbitrary so that we have no knowledge that it is a true subset of T. Such set fulfills the conditions of Theorem 5.

This completes the proof of the equation $2^{\aleph_0} = \aleph_1$.

ON THE EXISTENCE OF A CLOSED CONVEX SURFACE REALIZING A GIVEN RIEMANNIAN METRIC

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Let σ_0 be the surface of the unit sphere and $(ds_0)^2$ the first fundamental form of Differential Geometry on σ_0. Denote by $(ds_1)^2$ another given positive definite form defined at each point of σ_0 which in local parameters (u, v) reduces to

$$(ds_1)^2 = E(du)^2 + 2Fdudv + G(dv)^2.$$

Suppose that the Gaussian curvature K_1 of the metric $(ds_1)^2$ is positive. Weyl[1] has attacked the following problem: Does there exist a closed convex surface σ_1 which may be mapped in a one-to-one way on σ_0 so that the first fundamental form of σ_1 is precisely $(ds_1)^2$? (Embedment problem in the large.)

Weyl shows first that, under certain conditions affecting the differentiability of E, F, G, one can indicate a family of metrics $(ds_\lambda)^2$, $0 \leqq \lambda \leqq 1$,

$$(ds_\lambda)^2 = E_\lambda d(u)^2 + 2F_\lambda du dv + G_\lambda (dv)^2$$

in the parameters (u, v) on σ_0, such that

i) E_λ, F_λ, G_λ depend analytically on λ;
ii) for all λ the Gaussian curvature K_λ of $(ds_\lambda)^2$ is positive everywhere on σ_0;
iii) for $\lambda = 0$ and $\lambda = 1$, $(ds_\lambda)^2$ reduces to $(ds_0)^2$, respectively $(ds_1)^2$.

Weyl then proves: If there exists a surface σ_{λ_0} which solves the embedment problem of $(ds_{\lambda_0})^2$ for a certain value λ_0 of λ, then there also exists a sufficiently small neighborhood of λ_0, say $| \lambda - \lambda_0 | < \epsilon(\lambda_0)$, such that the embedment can be obtained for all λ of this neighborhood.

These facts alone don't permit to conclude the existence of a σ_λ for $\lambda = 1$, as the described continuity method does not exclude the possibility (π) that there may be a value λ^1 which cannot be reached from $\lambda = 0$ in finitely many steps. Weyl discusses this point in the second part of his paper, suggesting a line of attack and establishing certain estimates which he believes to be the clue to the solution. However these ideas have never been carried through.

I have been able by a different method[2] to eliminate (π) and thus to complete the solution of the embedment problem in the large. The following lines give the essential steps of the proof for the case of analytic E, F, G; in this case E_λ, F_λ, G_λ are analytic in u, v, λ.

A result of Darboux[3] shows that the distance $\rho_\lambda(u, v)$ from a fixed point P of the space to the point (u, v) of σ_λ satisfies a Monge-Ampère equation whose coefficients depend on E_λ, F_λ, G_λ and their derivatives as well as on ρ_λ, $\frac{\partial \rho_\lambda}{\partial u}$ and $\frac{\partial \rho_\lambda}{\partial v}$. The equation is elliptic because $K_\lambda > 0$. Take P as the center of the largest sphere inscribed to σ_λ. We want to prove: Let $0 \leq \lambda_1 < \lambda_2 < \ldots$ be a sequence tending to λ^1, such that $(ds_{\lambda_1})^2$, $(ds_{\lambda_2})^2$, ... can be embedded. Then $(ds_{\lambda^1})^2$ can be embedded. This fact is established as soon as we know that in the sequence ρ_{λ_1}, ρ_{λ_2}, ... of analytic functions of (u, v) there exists a subsequence converging to an analytic limit function $\rho_{\lambda^1}(u, v)$ which solves Darboux's equation for $\lambda = \lambda'$. This fact follows immediately from Theorem 2 of my paper[4] if the conditions of the theorem can be verified. They are:

i) $|\rho_{\lambda_1}|, |\rho_{\lambda_2}|, \ldots$ are uniformly bounded. This is a consequence of Bonnet's theorem which states that the diameter of a closed convex surface is bounded from above by a number depending only on a (positive) lower bound of the Gaussian curvature.

ii) $\left|\frac{\partial\rho_{\lambda_1}}{\partial u}\right|, \left|\frac{\partial\rho_{\lambda_1}}{\partial v}\right|, \left|\frac{\partial\rho_{\lambda_2}}{\partial u}\right|, \ldots$ are uniformly bounded. This follows from $(d\rho_\lambda)^2 \leqq (ds_\lambda)^2$.

iii) The coefficient of $\frac{\partial^2\rho_\lambda}{\partial u^2}\frac{\partial^2\rho_\lambda}{\partial v^2} - \left(\frac{\partial^2\rho_\lambda}{\partial u\partial v}\right)^2$ in Darboux's equation can be assumed to be $= 1$ while the other coefficients are bounded for complex values of u, v, ρ_λ, $\frac{\partial\rho_\lambda}{\partial u}$, $\frac{\partial\rho_\lambda}{\partial v}$ in a bounded neighborhood of their values under consideration. The contrary assumption is ruled out by a simple geometrical consideration showing that the radii of the maximum spheres inscribed in $\sigma_{\lambda_1}, \sigma_{\lambda_2}, \ldots$ are bounded away from zero.

iiii) Elimination of ρ_λ. The independent variables (u, v) considered as functions of variables (α, β) which are characteristic with respect to the Monge-Ampère equation, satisfy two equations of the form

$$\frac{\partial^2 u}{\partial\alpha^2} + \frac{\partial^2 u}{\partial\beta^2} = h_{1\lambda}\left[\left(\frac{\partial u}{\partial\alpha}\right)^2 + \left(\frac{\partial u}{\partial\beta}\right)^2\right] + h_{2\lambda}\left(\frac{\partial u}{\partial\alpha}\frac{\partial v}{\partial\alpha} + \frac{\partial u}{\partial\beta}\frac{\partial v}{\partial\beta}\right) + h_{3\lambda}\left[\left(\frac{\partial v}{\partial\alpha}\right)^2 + \left(\frac{\partial v}{\partial\beta}\right)^2\right] + h_{4\lambda}\frac{\partial(u, v)}{\partial(\alpha, \beta)},$$

$$\frac{\partial^2 v}{\partial\alpha^2} + \frac{\partial^2 v}{\partial\beta^2} = k_{1\lambda}\left[\left(\frac{\partial u}{\partial\alpha}\right)^2 + \left(\frac{\partial u}{\partial\beta}\right)^2\right] + \ldots + k_{4\lambda}\frac{\partial(u, v)}{\partial(\alpha,\beta)}, \quad (1)$$

where $h_{1\lambda}, h_{2\lambda}, \ldots, k_{1\lambda}, k_{2\lambda}, \ldots$ depend on (u, v), but not explicitly on ρ_λ nor $\frac{\partial\rho_\lambda}{\partial u}$ nor $\frac{\partial\rho_\lambda}{\partial v}$. In our case $\alpha + i\beta$ and $\alpha - i\beta$ are parameters on the asymptotic lines and our equations (1) are identical with equations already found by Darboux.[5]

[1] Hermann Weyl, "Über die Bestimmung einer geschlossen konvexen Fläche durch ihr Linienelement." *Vierteljahrsschrift der naturf. Ges. Zürich*, **61**, 40–72 (1915).

[2] I have been in possession of this proof for several years, cf. *Bull. Am. Math. Soc.*, **42**, 824 (1936). The preparatory investigations of Monge-Ampère equations were undertaken mainly for this purpose and published in *Trans. Am. Math. Soc.*, **37**, 417–434 (1935) and **41**, 365–374 (1937).

[3] G. Darboux, *Théorie des surfaces*, vol. 3, p. 260 (Paris, 1894).

[4] Loc. cit., **41**, 373 (1937); also *Bull. Am. Math. Soc.*, **42**, 689–692 (1936).

[5] Loc. cit., p. 290.

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